PROGRESS TOWARD THE SYNTHESIS OF (+)-DIBROMOPHAKELLIN AND CONGENERS: PROPOSED FINAL STAGES FOR PALAU'AMINE SYNTHESIS

A Thesis

by

FRANCISCO MIGUEL FRANCO-TORRES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2007

Major Subject: Chemistry

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Approved by:

Chair of Committee,	Daniel Romo
Committee Members,	Daniel Singleton
	Coran Watanabe
	J. Martin Scholtz
Head of Department,	David H. Russell

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ABSTRACT

Progress Toward the Synthesis of (+)-Dibromophakellin and Congeners:

Proposed Final Stages for Palau'amine Synthesis. (May 2007)

Francisco Miguel Franco-Torres, B.S., Universidad de Puerto Rico

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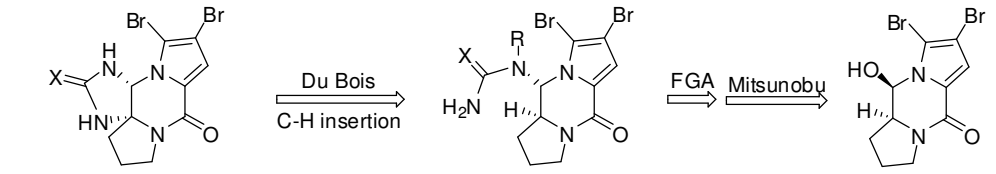
Chair of Advisory Committee: Dr. Daniel Romo

The pyrrole-imidazole alkaloid family of natural products illustrates the diversity of topographically unique molecules with potent biological activities that can be found in the marine environment. Thus, great interest for this class of compounds has developed leading to new synthetic methodologies and tactics to build these complex secondary metabolites.

The overall objectives of this research project include the total synthesis of the phakellins and phakellstatins. First, we revisited the strategy developed in our group for the total synthesis of (+)-dibromophakellstatin and utilized it for the synthesis of its naturally occurring enantiomer and congeners. Second, we proposed and studied a new and more concise approach to the phakellstatins and phakellins based on a key C-H insertion process developed by Du Bois.



X = NH: dibromophakellin
X = O: dibromophakellstatin

X = NH: pendant guanidine
X = O: pendant urea

Attempts to streamline the first synthesis of (+)-dibromophakellstatin proved to be quite challenging. Shortcomings in the reproducibility of some parts of the original strategy precluded us from completing and making more efficient the synthesis of the natural enantiomer (-)-dibromophakellstatin. Fortuitously, our second generation approach though it presented itself as an efficient route to the phakellins and phakellstatins produced the undesired *anti* diastereomer of the key guanidine intermediate which precluded our study of the pivotal C-H insertion reaction.

DEDICATION

To my parents, Francisco Franco Dominicci and Evette Torres Cintrón.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Daniel Romo for allowing me to work on such a challenging and rewarding project. I am also thankful to my committee members, Dr. Daniel Singleton, Dr. Coran Watanabe, and Dr. J. Martin Scholtz for their support throughout the course of this project.

I have enjoyed my time here at Texas A&M with the members of the Romo group, both past and present, as well as with my friends in other groups, inside and outside of the chemistry department. I would like to thank my mentors in the Romo group, Dr. Karine G. Poullennec and Dr. Paul J. Dransfield, for their friendship, excellent instruction in laboratory technique, and chemistry knowledge that certainly helped me through these past four years.

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CHAPTER I

AN INTRODUCTION TO THE SYNTHETIC AND BIOLOGICAL STUDIES OF THE TETRACYCLIC PYRROLE IMIDAZOLE ALKALOIDS PHAKELLINS, PHAKELLSTATINS, AND RELATED ISOLATES

A. Isolation and Biological Activity

As the cradle of mankind, the natural world not only supports people's needs, but also provides many novelties for study by organic chemists. Every year, a considerable number of alkaloidal secondary metabolites are isolated from natural sources; some of these belong to known frameworks but others are completely new. Several of these natural products have been extensively investigated because of their varying and often potent biological activities. Many have lured chemists to pursue their total synthesis and the evaluation of their potential as chemotherapeutic agents.[1] In fact, many of the anti-HIV drugs (75%) and anti-cancer drugs (62%) currently used in chemotherapy either are derived directly from natural products or are derived from natural products leads (about 52% of total drugs).[2] Natural products that have potent and specific cellular effects have proven to be extremely useful as biochemical probes for dissecting molecular mechanisms of signal transduction pathways involved in various cellular functions.

Marine organisms are known producers of compounds with novel chemical structures and interesting biological activities. The marine environment has proven to be a very rich source of extremely potent compounds that have demonstrated significant

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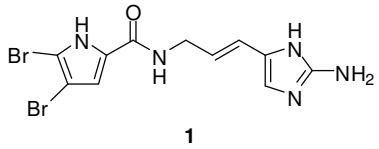
This thesis follows the style and format of the *Journal of the American Chemical Society*.

activities in anti-tumor, anti-inflammatory, analgesia, immunomodulation, allergy, and anti-viral assays.[3] Among all the marine organisms studied to date, sponges have been, and still are, the most investigated for their incredible metabolite production.[4] One class that are thought to be specifically derived from marine sponges and not symbiotic bacteria are the pyrrole-imidazole alkaloids. These alkaloids are an interesting family of natural products. An impressive number of these alkaloids have been isolated from different species of various families, mainly the *Agelisidae*, *Axinellidae*, and *Hymeniacidonidae*.[5] The components of this family are fascinating due to their biogenetic relationship leading to a large, structurally diverse family presumed to be derived from a common key biosynthetic precursor, oroidin (**1**) (Figure 1).[6]



1

Figure 1. Structure of oroidin (**1**), key metabolite to the pyrrole-imidazole alkaloids.

This family of alkaloids is composed of a diverse and complex class of bioactive secondary metabolites and only a few studies regarding their molecular mechanism of action have been reported.[7] Novel examples with unprecedented molecular architecture and biological activities are constantly being discovered. By the end of 2003, about 90 pyrrole-imidazole alkaloids had been characterized, some of which occur in high concentrations of more than 1% of the sponge dry weight, or as low as 10^{-4}%.[8] Within

the family of the pyrrole-imidazole alkaloids are the phakellins, shown in Figure 2; these are cyclized monomers of oroidin (**1**).

2a: $R_1 = R_2 = Br$: (-)-Dibromophakellin
2b: $R_1 = H$, $R_2 = Br$: (-)-Monobromophakellin
2c: $R_1 = R_2 = H$: (-)-Phakellin

3a: R = Br, (-)-Dibromophakellstatin
3b: R = H, (-)-Phakellstatin

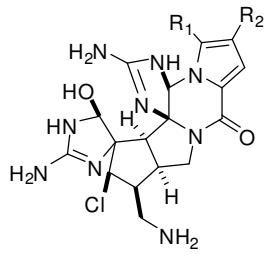
Figure 2. Structures of the phakellins and phakellstatins.

(-)-Dibromophakellin (**2a**) has been isolated from different sponges over different oceans. Sharma and Burkholder first isolated it in 1969, along with (-)-monobromophakellin (**2b**) and an unidentified antibacterial agent, from the marine sponge *Phakellia flabellata* at the Great Barrier Reef in Australia.[9] Dibromophakellin (**2a**) has also been isolated from *Phakellia mauritiana*, alongside with (-)-dibromophakellstatin (**3a**), off the coast of the Republic of Seychelles.[10] It has also been isolated from the Fijian sponge *Agelas mauritiana*,[11] from *Stylotella aurantium* in the Rock Islands, Republic of Belau,[12] and from the algae *Laurencia majuscula Lucas* collected from the China Sea.[13] Furthermore, (+)-dibromophakellin, the opposite enantiomer of **2a**, was isolated from the marine sponge *Pseudoaxinyssa cantharella* garnered off the coast of New Caledonia.[14]

The structures of the brominated phakellins were elucidated by extensive analysis including NMR spectroscopy, IR spectroscopy, UV spectrophotometry, mass

spectrometry, and confirmed by X-ray analysis of the monoacetyl derivative of (-)-dibromophakellin (**2a**). These compounds, **2a** and **2b**, exhibit pK_a values of < 8, which are rather low when compared to reported values for other guanidines with $pK_a > 13.4$.[9c] This could be explained in terms of *I*-strain concepts.[15] Theoretically, the basicity of a cyclic guanidine derivative will fall off rapidly due to departure from planarity of the CN_3 skeleton that in the case of the phakellins will be due to the twisted conformation of the imidazoline ring, which will prevent delocalization of the pyrrole nitrogen lone pair into the guanidine moiety.

The phakellins possess several exotic features, among them we can mention the fact that the cyclic guanidine has the double bond in an endocyclic position, a feature rarely observed in this kind of moiety. Also, both diaminoacetal (C6) and diaminoketal (C10) functionalities in the brominated phakellins are considerably stable toward hydrolytic agents. Dibromophakellin (**2a**) and monobromophakellin (**2b**) were isolated as the hydrochloride salts from *P. flabellata* and they have shown mild antibacterial activity against *Bacillus subtilis* and *Escherichia coli* and mild antineoplastic activity (see Table 1, *vide infra*).[9c] The responsible agent for the potent antibiotic activity in the crude methanol extracts of the sponge is due to some other substance(s) which has (have) not been identified yet.[9c] Although (-)-phakellin (**2c**) has never been isolated from a natural source, it was semi-synthetically synthesized and until fairly recent the greater activity for the methanol extracts from *P. flabellata* had been tentatively attributed to phakellin based on the observation that palau'amine (**4a**) is more potent than its brominated derivatives (Figure 3).[12]



4a: $R_1 = R_2 = H$: Palau'amine
4b: $R_1 = Br$, $R_2 = H$: Monobromopalau'amine
4c: $R_1 = R_2 = Br$: Dibromopalau'amine

Figure 3. Structures of the palau'amines.

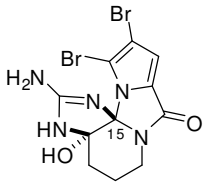
In 1997, Pettit and co-workers reported the isolation of (-)-dibromophakellstatin (**3a**), which differs from (-)-dibromophakellin (**2a**) by the replacement of the guanidine moiety for a urea, from *Phakellia mauritiana* as part of a bioassay-guided isolation procedure using human tumor cell lines (Figure 2, *vide supra*). In fact, dibromophakellstatin (**3a**) was the most significant cancer cell growth inhibitory substance obtained from the natural source (Table 1).

Table 1. Cell growth inhibitory activity of dibromophakellin (**2a**) and dibromophakellstatin (**3a**) against a mini-panel of human cancer cell lines.

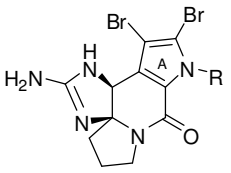
Human Cancer Cell Line	**ED_{50} (µg/mL)**	
	Dibromophakellin	**Dibromophakellstatin**
Ovary, OVCAR-3	15.7	0.46
Brain, SF-295	18.8	1.5
Kidney, A498	17.8	0.21
Lung, H460	22.0	0.62
Colon, KM20L2	20.1	0.11
Melanoma, SK-MEL-5	17.0	0.11

The structure of dibromophakellstatin (**3a**) was elucidated by NMR spectroscopic analysis (^{1}H and ^{13}C) comparison with dibromophakellin which resulted to be very similar and the molecular formula was deduced by HRFAB-MS. The absolute stereochemistry was determined by single crystal X-ray analysis taking advantage of the pronounced anomalous dispersion effects induced by the bromine atoms.

A very interesting bromine-containing guanidine derivative from the C_{11}-N_5 family, another way to refer to cyclized oroidin (**1**) monomers, was isolated from an *Agelas sp.* sponge in the coasts of Tanzania. The novel dibromopyrrole derivative was named dibromoagelaspongine (**5**) and while being a typical representative for the pyrrole-imidazole alkaloids, possesses a different molecular skeletal structure though it seems to be biogenetically related to the phakellins (Figure 4). Unambiguous elucidation of the structure of dibromoagelaspongine was achieved by X-ray analysis which revealed the highly uncommon *ortho*-imidine functionality at C15.



5: Dibromoagelaspongine



6: R = H, (-)-Dibromoisophakellin
7: R = CH_3, (-)-*N*-methyldibromoisophakellin

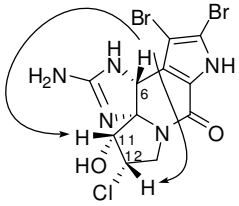
Figure 4. Structures of dibromoagelaspongine (**5**), the isophakellins (**6** and **7**).

Previous to the isolation of dibromophakellstatin (**3a**), Maximov and co-workers described the isolation of (-)-dibromoisophakellin (**6**) from *Acanthella carteri* collected

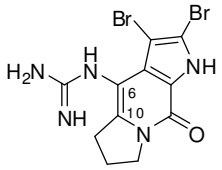
off Madagascar (Figure 4).[16] Careful spectroscopy analysis led to its characterization and it was found to be an A-ring isomer of dibromophakellin (**2a**). The proposed structure was confirmed by crystallographic analysis. Actually, the opposite enantiomer of dibromoisophakellin (**5**) was previously isolated from *Pseudaxinyssa cantharella* and named (+)-dibromocantharelline.[14] Dibromoisophakellin (**6**), like its structural isomer dibromophakellin (**2a**), has been isolated from variety a marine sponges alongside with plethora of natural products, some known and some new. One of those new metabolites is (-)-*N*-methyldibromoisophakellin (**7**) isolated from *Stylissa caribica* collected off the coast of Sweetings Cay, Bahamas (Figure 4).[17] Bioassay-guided fractionation of the methanol extracts of this sponge looking for antifeedant properties resulted on the isolation and structural elucidation of isophakellin **7**. Its absolute configuration was determined by comparison of the optical rotation of *N*-methyldibromoisophakellin (**7**) with that of dibromoisophakellin (**6**).[16] Interestingly, *N*-methyldibromoisophakellin (**7**) was shown to be the only secondary metabolite in *Stylissa caribica* that, at its natural concentration (0.9 mg/mL), is active as a feeding deterrent against a common omnivorous Caribbean reef fish, *Thalassoma bifasciatum*.

From the marine sponge *Axinella brevistyla* collected in western Japan was isolated 12-chloro-11-hydroxydibromoisophakellin (**8**) along with the known dibromoisophakellin (**6**) and other bromopyrrole alkaloids (Figure 5).[18] A positive FABMS of isophakellin **8** displayed a 1:2:1.5:0.4 ion cluster at *m/z* 438/440/442/444, indicating the presence of a chlorine and two bromine atoms. Its molecular formula of $C_{11}H_{11}Br_2ClN_5O_2$ was obtained by HRFABMS. COSY and HMBC experiments helped

in deciphering some of the key carbon-carbon connectivities on the molecule and NOESY cross-peaks from H6 to H11 and H12 suggested the relative stereochemistry of 12-chloro-11-hydroxydibromoisophakellin (**8**) as shown in Figure 5. 12-chloro-11-hydroxydibromoisophakellin (**8**) not only posses interesting skeletal features but also biological activities; in antifungal studies it showed growth inhibition of the *erg6* mutant of the yeast *Saccharomyces cerevisiae* at 100 μg/disk and also proved to be cytotoxic against L1210 cells with an IC_{50} value of 2.5 μg/mL.



8: 12-Chloro-11hydroxydibromoisophakellin



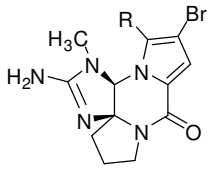
9: Ugibohlin

Figure 5. Structures of 12-chloro-11-hydroxydibromoisophakellin (**8**) and ugibohlin (**9**).

Another member of the $C_{11}N_5$ family that was isolated along with dibromoisophakellin (**6**) is ugibohlin (**9**) (Figure 5).[19] Isolated from *Axinella carteri* collected on a reed slope of Talakanen Island, Phillipines by Goetz and co-workers, ugibohlin (**9**) showed similar ^{1}H and ^{13}C NMR data to those of known compound dibromoisophakellin (**6**) and its enantiomer, dibromocantharelline.[14,16] The ^{1}H NMR spectrum of ugibohlin (**9**) lacked the methine signal (H6) found in the corresponding dibromoisophakellin (**6**) and dibromocantharelline. This piece of evidence along with

the presence of two extra nonprotonated sp^2 signals on the ^{13}C NMR spectrum implied that ugibohlin (**9**) was the C10-N9 seco-derivative of dibromoisophakellin (**6**) or dibromocantharelline and that the C6-C10 bond was unsaturated.

Two new members of the phakellin family were reported by Crews and co-workers, (-)-7-*N*-methyldibromophakellin (**10a**) and (-)-7-*N*-methylmonobromophakellin (**10b**), and their structures are shown in Figure 6.[20] They were isolated from an undescribed *Agelas sp.* sponge collected near Wewak, Papua New Guinea, using a combination of bioassay- and LCMS-guided fractionation. Their structure elucidation was completed by spectroscopic data analysis and comparison to the properties of the known phakellins. The guided isolation assays were done employing both 12- and 15-human lipoxygenase isozymes (12-HLO, 15-HLO). (-)-7-*N*-methyldibromophakellin (**10a**) exhibited moderate selectivity towards 12-HLO (12-HLO IC_{50} = 10.7 ± 1.3 μM; 15-HLO IC_{50} = 48 ± 16 μM). 12-HLO isozymes have been implicated in psoriasis and cancer cell proliferation *via* its role in the production of leukotrienes and lipoxins.[21] (-)-7-*N*-methylmonobromophakellin (**10b**) was not tested due to small sample amount.



10a: R = Br : (-)-7-*N*-methyldibromophakellin
10b: R = H : (-)-7-*N*-methylmonobromophakellin

Figure 6. Structures of the *N*-methylbromophakellins.

Recently, Al-Mourabit and co-workers isolated four new $C_{11}N_5$ metabolites from the Mediterranean sponge *Axinella vaceleti*.[22] The verpacamides A-D, as they were named, are interesting bi- and tricyclic secondary metabolites which could potentially be derived from proline and arginine (i.e. verpacamide A) and further metabolized into the other verpacamides (Figure 7). Verpacamides C (**13**) and D (**14**) are quite unique since these are the first examples of proline-proline-guanidine structures reported from marine sponges. If verpacamides C and D are intriguing, even more so is the natural ring closure of the arginine in verpacamide B (**12**) into a guanidine-bearing proline functionality in verpacamide C (**13**) observed on this metabolite for the first time. Proline and arginine biosynthetic interconversion was only known *via* an ornithine intermediate.

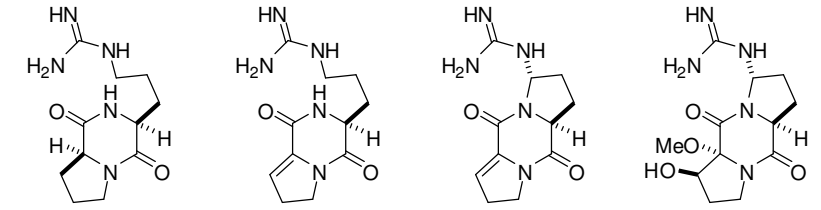


Figure 7. Structures of the verpacamides A-D.

B. Biosynthetic Pathways

The biosynthetic machinery found in natural product producers has used very subtle chemistry based on the reactivity variation of a single precursor or a restricted number of precursors that under enzymatic-catalyzed primary and secondary metabolic

cycles produce a plethora of structural types. The fascinating variety of secondary metabolites from the pyrrole-imidazole alkaloid family is not the exception since it contains some of the most unprecedented and intriguing molecules isolated from marine sponges. Various speculative statements about the origin and mode of formation of the pyrrole-imidazole alkaloids have recently appeared in the literature.

1. Biosynthetic Proposals

Al-Mourabit and Potier have proposed a plausible biogenetic mechanism that might help clarify the chemical pathway leading to over 90 polycyclic pyrrole-imidazole alkaloids.[6] This hypothetical biogenetic pathway is an important step now that it may lead to new ideas for biomimetic total syntheses of this group of natural molecules. Within this group of alkaloids, it is easy to see that all derivatives are closely related, but still a logical chemical path to corroborate the presupposition of a common biogenesis is lacking. The authors propose a very likely universal chemical conduit through simple precursors such as 3-amino-1-(2-aminoimidazolyl)-prop-1-ene (**15**) and 4,5-dibromopyrrole-2-carboxylic acid (**16**) shown in Figure 8.

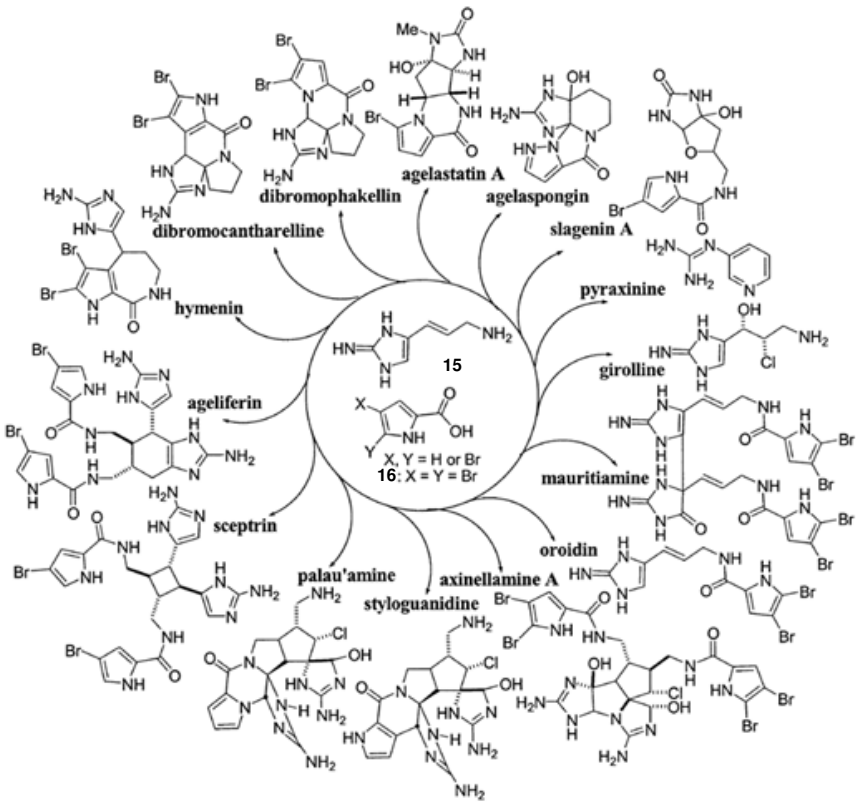
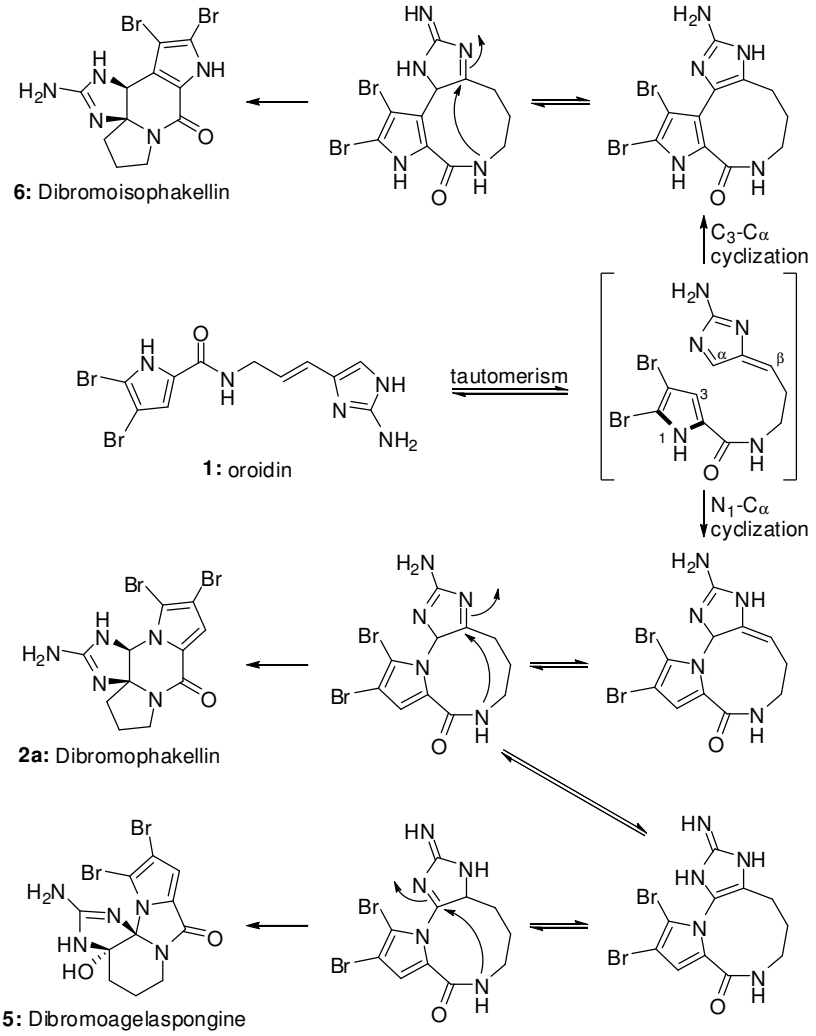


Figure 8. Representative secondary metabolites from the pyrrole imidazole alkaloids.

Within their universal chemical outline, Al-Mourabit and Potier depicted the variety of modes of intramolecular cyclization that linear compounds, i.e. oroidin (**1**), can undergo to overcome various assemblies of polycyclic natural derivatives. Illustrated in Scheme 1 are three cyclization modes for the formation of dibromophakellin (**2a**), dibromoisophakellin (**6**), and dibromoagelaspongine (**5**).

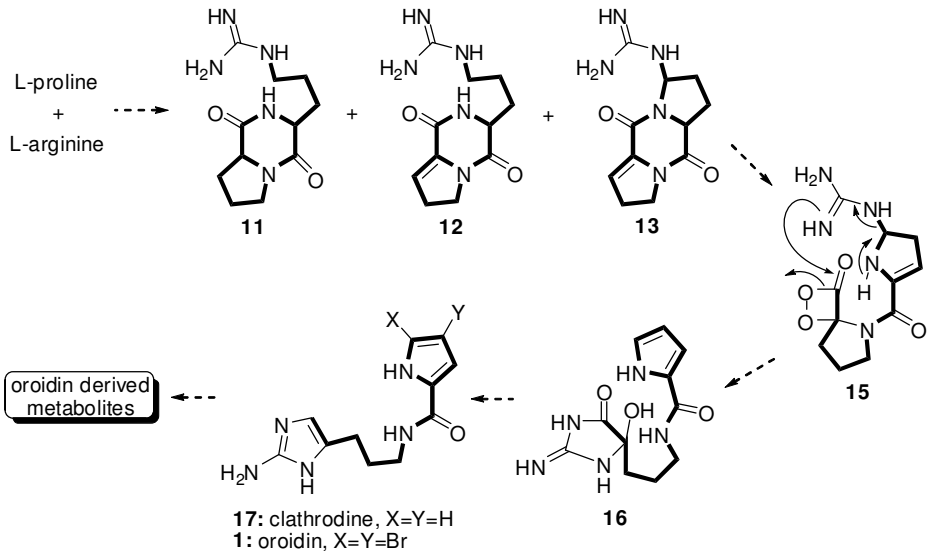
Scheme 1



Based on the recent discovery of the verpacamides A-D (Figure 7, *vide supra*), Al-Mourabit and co-workers felt that these metabolites could potentially be early biosynthetic precursors of $C_{11}N_5$ derivatives.[22] These diketopiperazines derivatives (**11**, **12**, or **13**) may be considered as a combination of proline and arginine or proline-proline and guanidine and the first oxidized members in the pathway to transform the proline skeleton into pyrrole and 2-aminoimidazole derivatives (Scheme 2). The supposed

cascade begins with the generation of cyclo(Pro-Arg) **11** and its further oxidation into a dioxetanone derivative such as **15**.[23] The driving force for the conversion to aminoimidazolone **16** would be the aromatization of one proline moiety into pyrrole and the intramolecular transfer of the guanidine. Further transformations would lead to the essential precursors clathrodine (**17**) and oroidin (**1**), which as mentioned before play an important role in the production of more complex pyrrole-imidazole alkaloids.

Scheme 2



2. Biosynthetic Studies

Biosynthetic investigations have been successful with plants and microorganisms by using cell cultures, however, this technique is particularly difficult to apply to marine invertebrates like marine sponges because of the intricacy on the establishment of viable and continuous cell lines.[24] Other problems associated with biosynthetic studies of

marine organisms are (1) the complex organic extracts produced from the marine organism where the components of interest are present in minute quantities; (2) the rate of synthesis *in vivo* of the relevant metabolites is low and conditioned to seasonal and environmental variations; (3) the construction of artificial environments for marine sponges poses technical difficulties since marine invertebrates are filter feeders thus in their natural environment they digest bacteria and other particulate that the sea current might bring; (4) marine organisms commonly form symbiotic associations in their natural habitat; (5) the uptake and transport of precursors relative to the concentration of nutrients could work against a concentration gradient and uptake might not occur; and (6) studies in natural environments are almost impossible to do due to the uncontrollable nutrient supply to the animals.[25] Despite all these factual experimental obstacles, Kerr and co-workers were able to establish reproducible primary cultures of *Teichaxinella morchella* archaeocytes to produce the bioactive metabolite stevensine (**18**).[24] Their subsequent incorporation experiments utilizing the radiolabeled amino acids ^{14}C-histidine, ^{14}C-arginine, ^{14}C-ornithine, and ^{14}C-proline showed that only ^{14}C-histidine, ^{14}C-ornithine, and ^{14}C-proline gave radioactive stevensine as shown by the percent incorporation of each amino acid in Table 2.

Table 2. Recovered radioactivity in stevensine (**18**).

Labeled Amino Acid	Recovered Radioactivity in **18**	% Incorporation in **18**
[U-^{14}C]histidine (5.5x10^6 dpm)	1460 dpm	0.026
[U-^{14}C]arginine (5.5x10^6 dpm)	Background	-----
[C_5-^{14}C]ornithine (5.5x10^6 dpm)	1300 dpm	0.024
[U-^{14}C]proline (5.5x10^6 dpm)	1180 dpm	0.022
control	background	-----

Based on the radioactivity experiment is that both proline and histidine are presented as the most likely natural precursors for stevensine **18** and possibly other pyrrole-imidazole derived alkaloids (Figure 9).

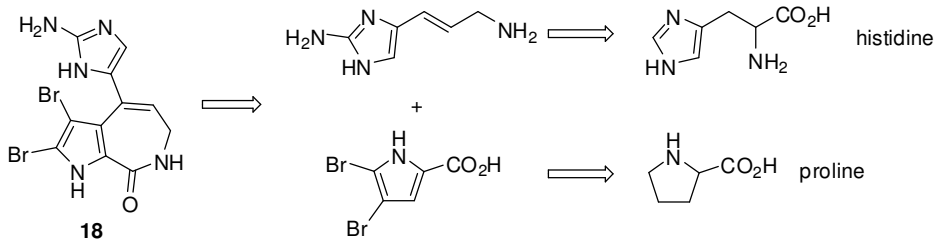


Figure 9. Biogenetic precursors of stevensine (**18**) from the sponge *T. morchella*.

C. Synthetic Studies

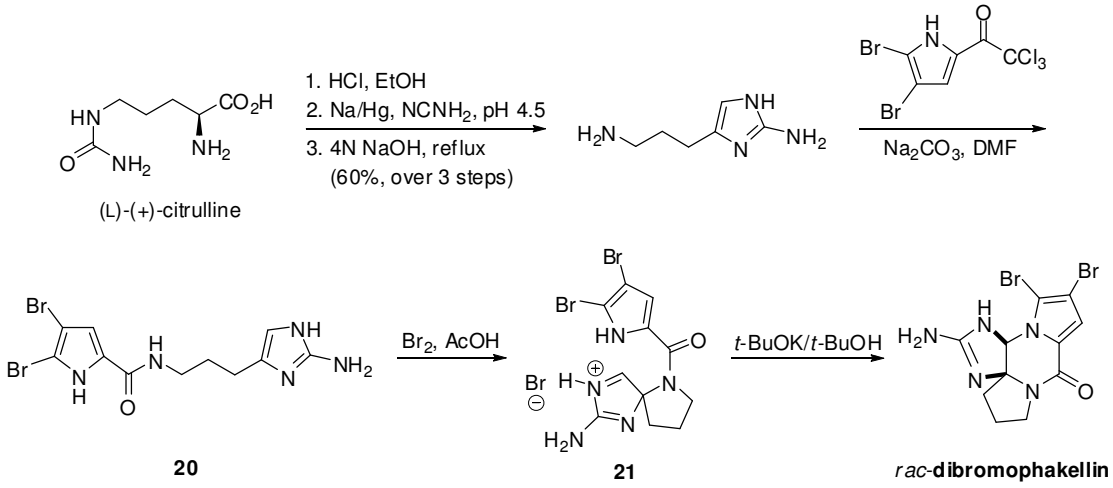
In the last few years, an increasing interest in the pyrrole-imidazole alkaloid family has triggered a surge in efforts toward the synthesis of these fascinating secondary metabolites. This is thought to be due to their widespread appearance in nature and their general biological activity (specific cellular targets for many of these molecules remain unknown), combined with their exquisite structures.

1. Biomimetic Approaches

The biomimetic synthesis of natural products requires highly selective reaction conditions that would trigger the desired mode of oxidation/cyclization, in the case of linear substrates like oroidin (**1**). Pioneering work in the area of pyrrole-imidazole alkaloids was reported by Foley and Büchi in 1982 where an elegant biomimetic

synthesis of (±)-dibromophakellin (**19**) was completed.[26] The key step in Büchi's loom is the oxidative bromination/cyclization of dihydro-oroidin (**20**) to the spiro-imidazolium intermediate **21** which upon treatment with potassium *tert*-butoxide in *tert*-butanol produced racemic dibromophakellin (**19**).

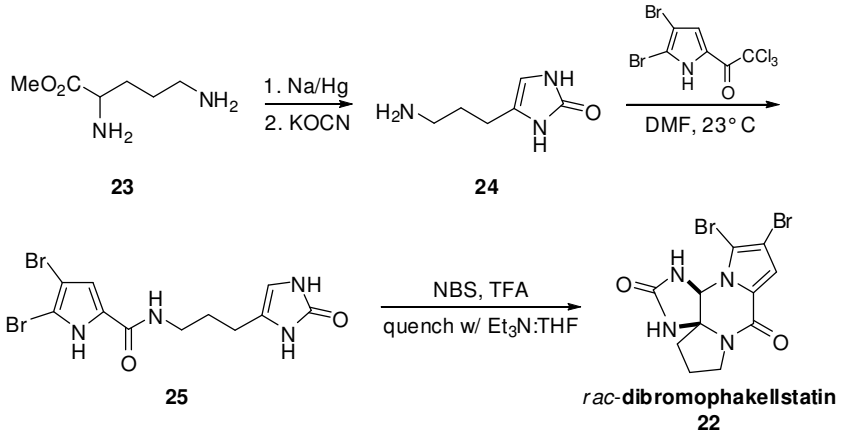
Scheme 3



Two decades later, in 2002, Horne and co-workers published the first racemic synthesis of (±)-dibromophakellstatin (**22**) and dibromoisophakellin /dibromocantharelline using a biomimetic oxidative cyclization protocol that sets the stage for construction of the tetracyclic framework.[27] In their approach towards dibromophakellstatin **21** they recognized aminoprolyl imidazolone **24** as a potential forerunner (Scheme 4). Imidazole **24** was readily prepared from ornithine methyl ester (**23**) *via* Akabori reduction and condensation with cyanate. After considerable experimentation, they found that oxidation of imidazolidinone **25** with NBS followed by

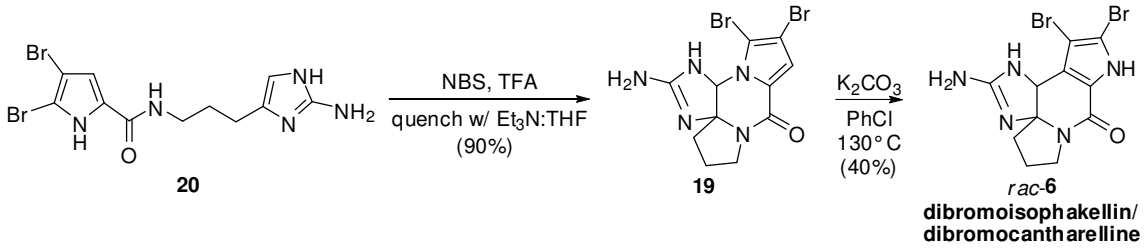
concentration and addition of triethylamine/THF produced racemic dibromophakellstatin (**22**).

Scheme 4



Next, in their attempts to synthesize dibromoisophakellin (*rac*-**6**) from dihydro-oroidin (**20**), Horne and co-workers discovered that their conditions previously used for racemic dibromophakellstatin **22** gave excellent yields of racemic dibromophakellin **19** (Scheme 5).[27] This represents a noteworthy improvement over the original Büchi conditions. Treatment of dibromophakellin **19** with K_2CO_3 in chlorobenzene at 130° C caused the N to C rearrangement to deliver dibromoisophakellin/dibromocantharelline along with recovered starting material. This rearrangement represents the first successful segway to the isophakellin series.

Scheme 5

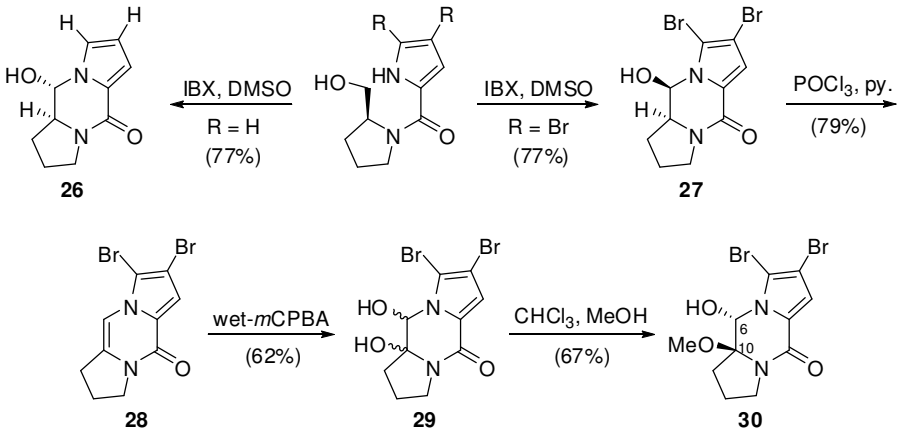


2. Synthetic Approaches to the Dipyrrolopyrazinone Core of the Phakellins and Phakellstatins

Towards the $C_{11}N_5$ metabolites, Lindel and co-workers showed that the relative stereochemistry of the carbinolamine formed on the dipyrrolopyrazinone core of the phakellins and phakellstatins (meaning the ABC ring system) depends on the bromination pattern of the pyrrole moiety.[28] Oxidation of the peptide coupling products of L-prolinol and pyrrole acid afforded exclusively *N*,*O*-hemiacetals **26** and **27** (Scheme 6). They found that the presence of a bromine substituent at C5 of the resulting tricycle exclusively favors the β-hydroxy tricycle **27**, possessing an *anti* relative configuration of the hydroxy group and the adjacent methine hydrogen. A possible rationale was proposed for this observation which is that the hydroxyl group adopts the axial position due to an anomeric effect induced by the pyrrole nitrogen when the pyrrole is not substituted. In the dibrominated derivative, the pyrrole moiety is electron poor thus the pyrrole nitrogen lone pair does not induce such a strong anomeric effect and the hydroxyl group occupies the more thermodynamically stable equatorial position on steric grounds. Interestingly, when diol **29** was exposed to mild acidic conditions only

one diastereomer was formed, methanol adduct **30**. This proves that the quaternary hemi-ketal C10 is stereochemically less stable than its tertiary hemi-acetal homolog C6 due to the ease of *N*-acyliminium formation.

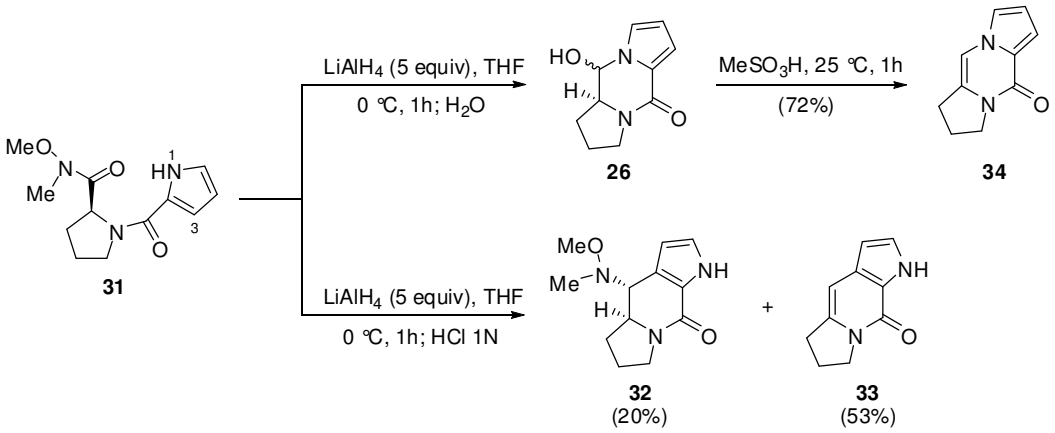
Scheme 6



Regioselective intramolecular cyclization studies on pyrrole-proline derivatives to deliver the ABC tricyclic core of the phakellins/phakellstatins and the isophakellins, for example, dibromoisophakellin (**6**) and ugibohlin (**9**), have been reported by Al-Mourabit and co-workers.[29] According to the authors, pyrrole N_1-C and C_3-C regioselective cyclization is closely dependent on the electrophilic function, bromination degree of the pyrrole moiety and pH conditions. After some exploratory attempts, intramolecular cyclization of Weinreb amide **31** was observed when treated with $LiAlH_4$ at 0° C (Scheme 7). Notably, the reaction led to pH dependent cyclization products; when the reaction was quenched with 1N HCl, the C_3-C cyclization product **32** along with enamine **33** was isolated. On the other hand, when the reaction was quenched with

just water only *N*,*O*-hemiacetal **26** was obtained. Al-Mourabit and co-workers rationalized the formation of **32** as being derived from the corresponding aldehyde after complete reduction and hydrolysis of the Weinreb complex.

Scheme 7



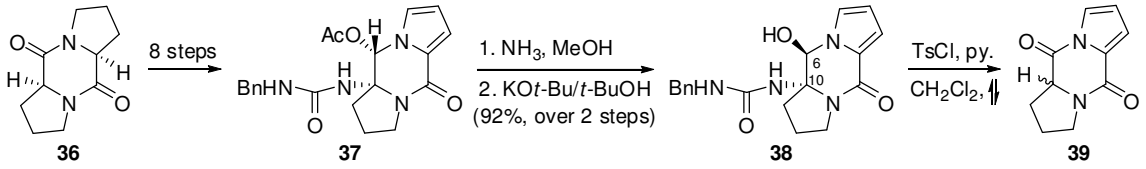
The authors confirmed that the reduction/cyclization of Weinreb amide **31** is totally masked by electronic effects of the bromine atoms since treatment of the dibromopyrrole derivative of Weinreb amide **31** under the reaction conditions above (reduction and hydrolysis with both 1N HCl and water) gave exclusively *N*,*O*-hemiacetal **27**.

3. Stereoselective Approach

The synthetic challenge presented by the monomeric polycyclic orodin derivatives lies on the assembly of heteroatom rich molecules and the construction of stereocenters that have proven to be chemically unstable such as carbinolamines and

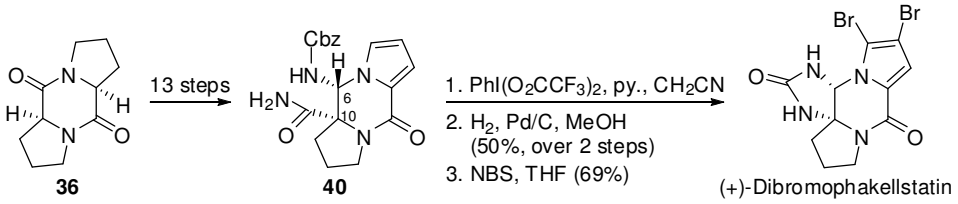
aminals. Poullennec and Romo took that challenge and were the first to report an enantioselective total synthesis of (+)-dibromophakellstatin (**35**), the antipode of the natural product **3a**.[30] As previously mentioned in Section A, this tetracyclic alkaloid not only possess exquisite structural features due to its compact size and corresponding dense polar functionality but also has interesting biological activity. They targeted the enantioselective synthesis of this alkaloid to enable biological investigations including mode of action studies. Moreover, the group's interest in the related natural product palau'amine (**4a**) provided further impetus for developing routes to access these unique tetracycles as they could reveal potential annulation strategies for the phakellin (**2c**) substructure embedded within palau'amine. Their strategy was premised on the observation that a prolyl-proline anhydride cyclo(Pro-Pro) was embedded within the structure of the natural product (Scheme 8). Hence this core may be accessed *via* desymmetrization of a C_2-symmetric diketopiperazine (DKP). Poullennec and Romo's approach began with (S,S)-cyclo(Pro-Pro) **36** obtained in three steps by a modified procedure from L-proline. Subsequent desymmetrization of the cyclo(Pro-Pro) *via* diastereoselective acylation, followed by dehydrogenation, then reduction and esterification which upon hydrogenolysis and treatment with Shioiri's reagent led to the formation of urea **37**. Aminolysis of urea **37** and subsequent epimerization delivered *trans*-carbinolamine **38** which failed to effect the desired cyclization when treated under a number of different conditions. The lability of C10 is presumably due to acyliminium chemistry. The instability of quaternary aminal centers compared to carbinolamines had previously been observed by Lindel on his work with dipyrrolopyrazinones.[28]

Scheme 8



Another strategy was taken to complete the synthesis of dibromophakellstatin **35** in which the C6 more stable aminal had to be introduced first followed by the more labile C10 aminal. β-amino amide **40** possess the desired properties and thankfully underwent the tandem Hoffman rearrangement/cyclization followed by hydrogenolysis to deliver (+)-phakellstatin, which upon bromination conditions with NBS delivered (+)-dibromophakellstatin (**35**) in 16 steps and 1.6% overall yield (Scheme 9).

Scheme 9

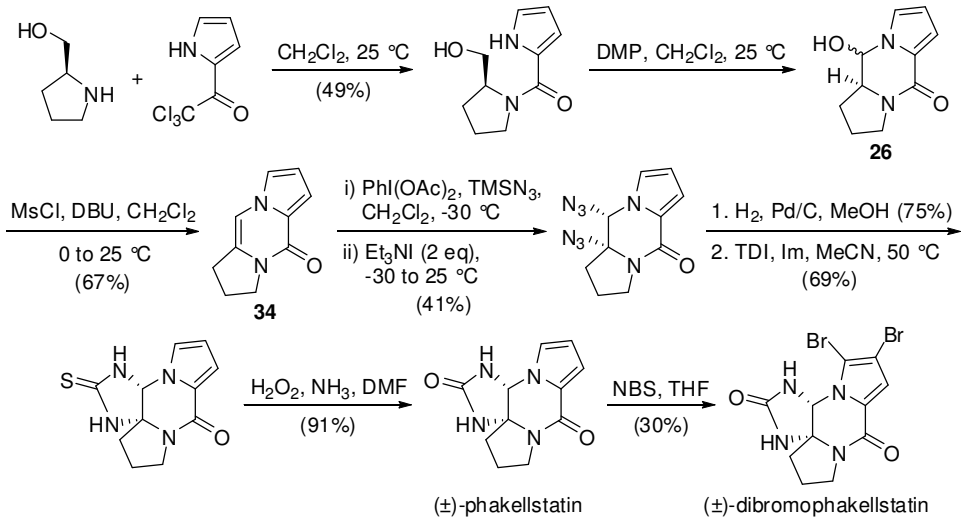


4. Racemic Approaches

In the last several years, a heave in publications regarding synthetic approaches toward the phakellstatins seems to signal a renewed interest in this class of marine alkaloids. Austin and co-workers took advantage of a key *syn*-diazide formation to construct both diamino acetal and diamino ketal centers onto the ABC core structure of

dibromophakellstatin.[31] Their approach utilizes a hypervalent iodine-mediated diazidation to assemble a diamine appropriate for the installation of the urea moiety on the dihydropyrrolopyrazinone (**34**) core structure of the natural product (Scheme 10).

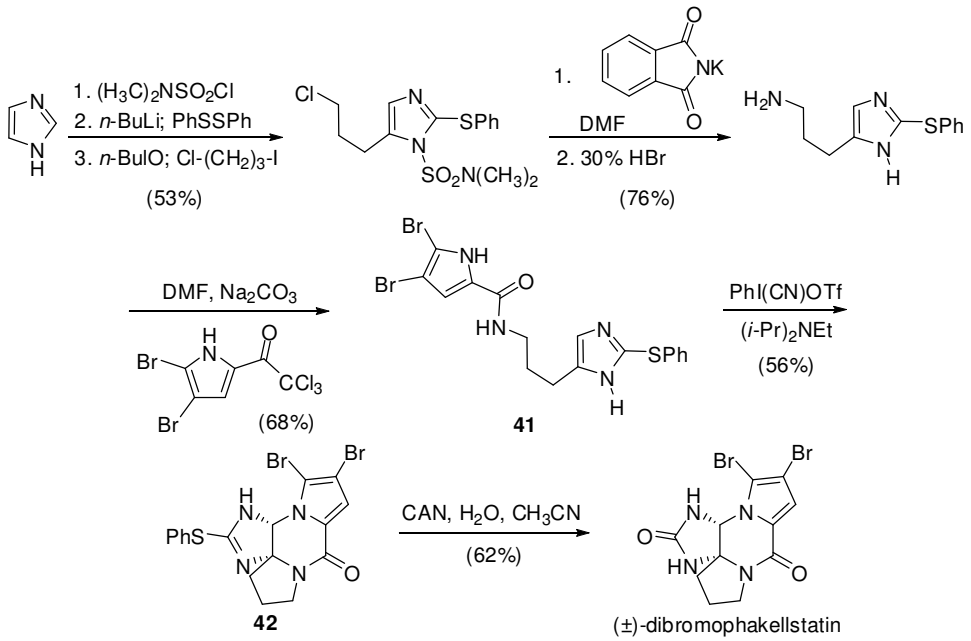
Scheme 10



Feldman and Skoumbourdis based their synthetic approach in mimicry the biosynthetic proposal of many polycyclic marine alkaloids where the oxidation of 2-aminoimidazole systems with concomitant cyclization of a tethered nucleophile leads to formation of their molecular framework.[32] Seminal work in this area by Büchi[26] and Horne[27] illustrate the value of this strategy for rapid access to variety of targets like dibromophakellstatin (**3a**) and dibromophakellin (**2a**). The authors utilized a variant of the Pummerer reaction to carry out a diastereoselective oxidative cyclization of a dihydrooroidin derivative (**41**). The thioimidate (**42**) product formed by reaction with

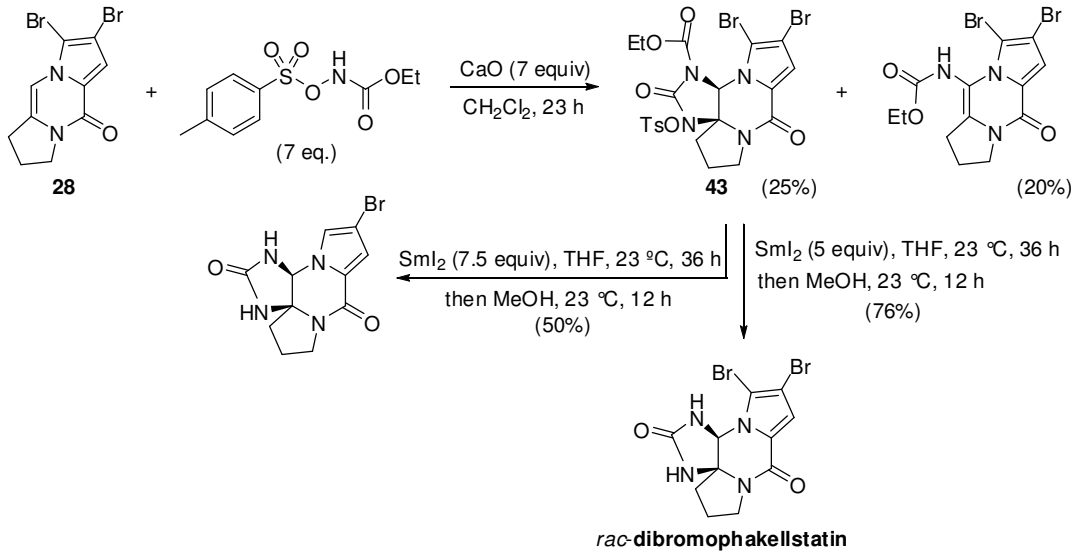
Stang's reagent, PhI(CN)OTf, served as a precursor to (±)-dibromophakellstatin (Scheme 11).

Scheme 11



Within a couple of months after Feldman's account, Lindel and co-workers also reported a racemic synthesis of dibromophakellstatin.[33] Lindel's strategy possess as key steps the direct assembly of imidazoline ring (cyclic urea) from the readily available unsymmetrical enamide **28** by means of nitrene chemistry and the simultaneous deprotection of the nitrogen atoms of the tetracycle **43** with samarium iodide (Scheme 12).

Scheme 12



D. Biological Studies

A precedent was set by Scheuer's[12] article in 1998, in which the question of whether Sharma's unidentified potent antibacterial activity might simply have been due to phakellin (**2c**). This hypothesis was premised by analogy to the findings regarding the brominated palau'amines being less active/potent than the non-brominated congener. If the hypothesis is true, and the phakellin substructure of palau'amine is necessary and possibly sufficient for its antimicrobial activity, this grants justification to attempt to synthesize phakellin. Harran and Nakadia motivated by this assertion decided to synthesize racemic phakellin (±-**2c**) and test its biological activity.[34] They used Horne's modifications[27] of Büchi's protocol to yield (±)-dibromophakellin (±-**2a**). Sharma's hydrogenolysis procedure was then employed to generate (±)-phakellin. When both compounds were screened against a variety of gram negative and gram positive bacteria,

as well as several drug efflux pump mutant strains, they were found to be devoid of activity. The authors claim that phakellin is not Sharma's missing active compound based on the assumption that one enantiomer does not antagonize the action of the other while being assayed together.

E. Conclusion

The pyrrole-imidazole alkaloid family of natural products illustrates the diversity of topographically unique molecules with potent biological activities that can be found in the marine environment. Not surprisingly, they have attracted a considerable amount of interest from the scientific community and are still the subject of intensive on-going research.

CHAPTER II

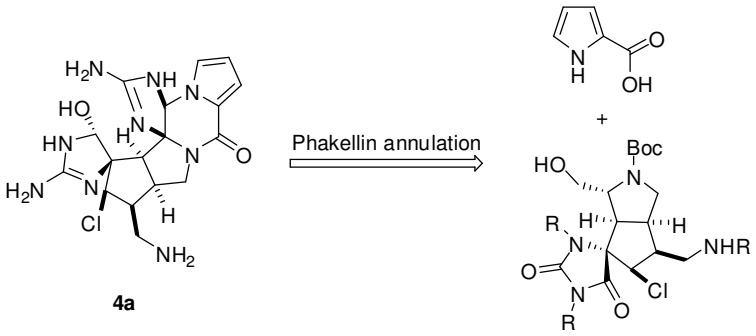
SYNTHESIS OF (-)-DIBROMOPHAKELLSTATIN: ORIGINAL STRATEGY REVISITED

The molecular complexity of the phakellins and phakellstatins poses a considerable synthetic challenge considering their compact, heteroatom dense tetracyclic core that includes a pyrrole, either a cyclic guanidine or a cyclic urea, and two vicinal, stereogenic aminal centers. These characteristics along with reported biological activities captured our attention and we targeted the asymmetric synthesis of these marine alkaloids. In addition, phakellin (**2c**) is a substructure within palau'amine (**4a**), which provides further impetus for the development of strategies to access this unique tetracycle in an efficient and concise manner since it could serve as a model study for phakellin annulation in the final stages of our proposed synthesis of palau'amine.

In our first asymmetric strategy toward these natural products, it was recognized that a bis-proline diketopiperazine (DKP) was embedded within their structure and thus a desymmetrization process of a C_2-symmetric DKP was utilized to construct these molecules. The first total synthesis of (+)-dibromophakellstatin (**35**), the unnatural enantiomer, which was reported by our group in 2003, proceeded through a potential, but not yet isolated, natural product (+)-phakellstatin.[30] The synthesis was 16 linear steps (from L-proline) and 1.6% overall and thus there is opportunity for optimization and improvement. As mentioned above, phakellin (**2c**) is embedded within the structure of palau'amine, therefore a new more concise strategy for its total synthesis will not only

improve the current strategy developed in our group for the total synthesis of (+)-dibromophakellstatin, but it will also provide a succinct annulation approach for the total synthesis of palau'amine (Scheme 12).

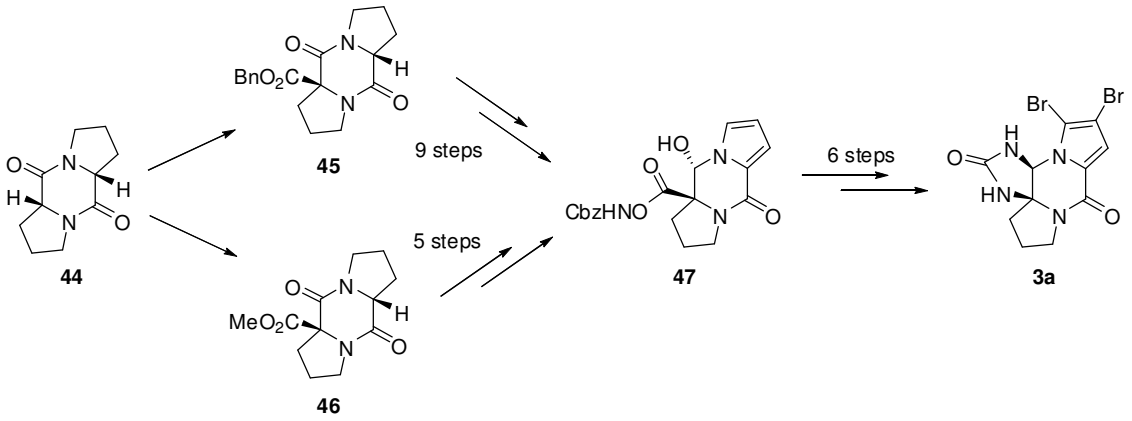
Scheme 12



A. Improving Efficiency

Towards improving our first total synthesis strategy toward these alkaloids and derivatives, we decided to streamline the linear sequence for the total synthesis of (+)-dibromophakellstatin (**35**). The new approach involved the use of carbomethoxy DKP-**46**, easily obtained by desymmetrization of the (-)-bis-proline DKP **44**. By using **46** as starting point it would take five steps to reach carbinolamine **47**, while the original sequence took nine steps to obtain the same intermediate **47** (Scheme 13).

Scheme 13



Carbinolamine **47** was an ideal intermediate to intercept in the synthesis since from this point the route could diverge to reach two goals. The first goal entailed following the original sequence to prepared monobrominated phakellstatin (**48**) (Figure 10). This mono-bromo derivative would be useful for mechanism of action studies (see Chapter III) by palladium (Pd) catalyzed couplings to provide cellular probes. The second goal would follow a similar sequence to access the isolation and characterization of an interesting by product, which was isolated by Dr. K. Poullennec and tentatively assigned as the C6-hydroxy phakellstatin **49**. Interestingly, the latter compound is structurally related to dibromoagelaspongine,[35] a natural product that contains a highly uncommon *ortho*-imidine moiety.

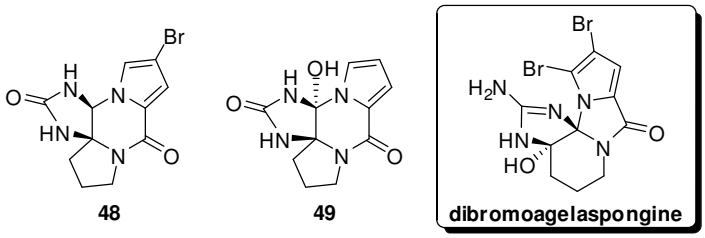
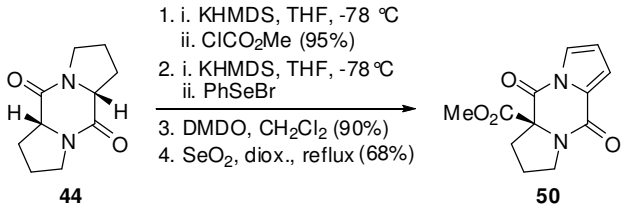


Figure 10. Structures of bromophakellstatin **48**, an interesting by-product **49**, and a structurally related natural product, dibromoagelaspongine.

1. Attempted Carboxylate Formation

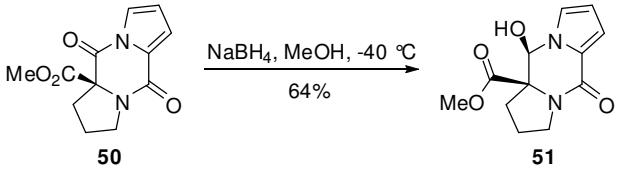
Preparation of the C_2-symmetric (-)-cyclo(Pro,Pro) DKP-**44** was accomplished following Yamamoto's procedure.[36] Desymmetrization of the DKP under conditions developed in our laboratory for these purposes[37] gave carbomethoxy DKP-**46** (Scheme 14). Dehydrogenation of the pyrrolidine to the pyrroline was accomplished following a selenation/oxidation/elimination sequence in good overall yield, even on multi-gram scale reactions. Final dehydrogenation of the pyrroline to provide the functionalized pyrrole **50** was efficiently obtained by means of selenium dioxide.

Scheme 14



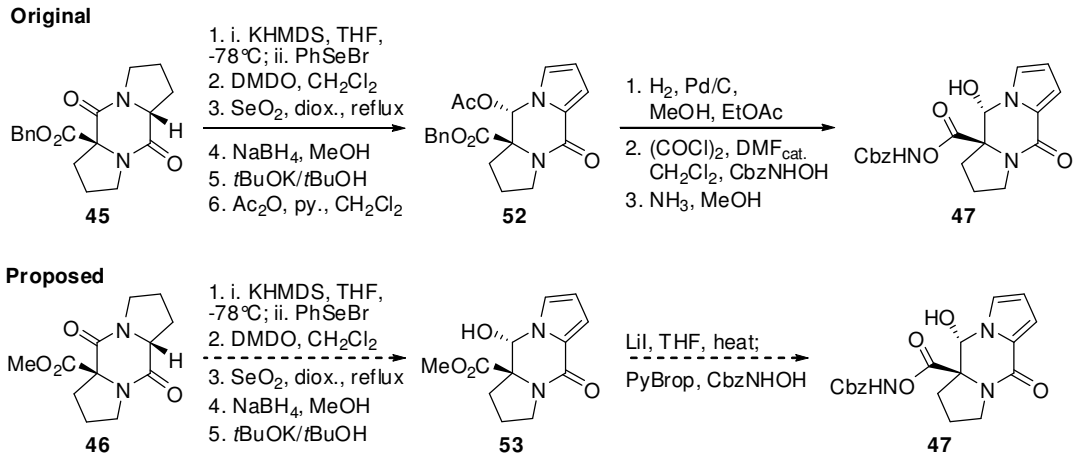
The reduction of the acyl pyrrole carbonyl in **50** with sodium borohydride at low temperature yielded the undesired *syn* carbinolamine **51** (Scheme 15).

Scheme 15



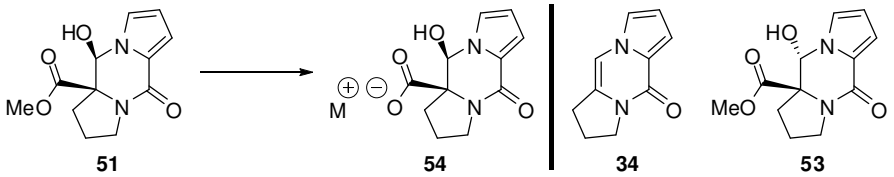
The stereochemical outcome of the reaction was realized only later when studying conversion to the carboxylate **54**. The idea for the carboxylate formation was based on that once this one was formed we could couple it with carbobenzyloxy hydroxylamine (CbzNHOH) by means of PyBrop and obtain hydroxamic ester **47** in four steps less than the original methodology (Scheme 16).

Scheme 16



Numerous conditions were tried in order to obtain carboxylate **54** but all failed (Table 3). Krapcho conditions were studied first using lithium iodide in an aprotic polar solvent, THF.[5] Theoretically, in the system there is a "pulling factor" *via* coordination with the lithium ion and a "pushing factor" from the nucleophile iodide ion both of which contribute to the carbon-oxygen bond cleavage. It is also important that the solvent possess a dielectric constant < 10.[5a] For this purposes the preferred solvents are ethyl acetate (ε = 6.0) and tetrahydrofuran (ε = 7.6); however, other solvents can be employed, for example, dichloromethane (ε = 8.9). It is proposed that the ability of the lithium ion to activate the carbonyl is lost if the solvent's dielectric constant > 10.

Table 3. Attempted carboxylate formation of **51**.



Entry	Conditions	Outcome
1	LiI, THF, reflux	Decarboxylation (**34**)
2	LiI, THF, 22 → 50° C	epimerized + unidentified
3	LiI, EtOAc, 22 → 50° C	epimerized + unidentified
4	LiI, CH_2Cl_2, 22 → reflux	epimerized + unidentified
5	LiOH, THF/H_2O, 22° C	epimerized + unidentified
6	LiI, THF, MeOH(cat.), 22 → 50° C	epimerized + unidentified
7	LiCl, DMF, 22° C	Multiple spots
8	KCN, DMF, 22° C	Multiple spots
9	KSCN, DMF, 22° C	Multiple spots
10	Amberlite, THF, 22° C	One spot (**53**)

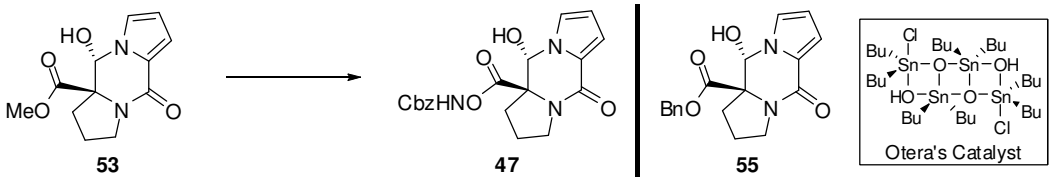
The product of entry 1 was identified as enamide **34** by direct comparison with published data for this compound (^{1}H NMR).[39] From that result we inferred that carbinolamine **51** might not be very stable to high temperature conditions. Changes in solvent and/or temperature and time of reaction did not prove beneficial (Entry 2-4, 6). Changes in the hardness of the nucleophile, iodide to chloride, or changing the cation for a soft ion like potassium and having soft nucleophiles like cyanide or thiocyanate, had no effect on the reaction outcome. Unexpectedly, amberlite gave a clean spot to spot reaction (Entry 10). The product of the reaction was identified as the diastereomer (α-hydroxy-**53**) of the starting material, epimerization of the alcohol had occurred under these reaction conditions.

2. Attempted Transesterification

Since demethylation proved more difficult than expected, we proposed an alternate route. A direct transesterification of hydroxy methyl ester **53** with *N*-hydroxycarbamate (CbzNHOH) using Otera's catalyst[40] which would remove the need for a protection/deprotection sequence used in our original strategy was sought. This strategy would deliver the Mitsunobu precursor **47** in four steps less than the original methodology. After various trials, varying time, equivalents of reagents, and catalyst loading, we finally were able to isolate one major product out of the reaction mixture, benzylester **55** (Table 4). We verified its identity by comparison with a known sample previously prepared by Dr. K. Poullennec.[39] Although the results compelled us, they were not totally surprising considering the fact that CbzNHOH is just a protected form

of benzyl alcohol. Classical transesterification conditions require that the alcohol partner be in excess relative to the ester. However, it appears that the propensity of the masked alcohol to undergo Lossen rearrangement leading to benzyl alcohol which then reacts under the reaction conditions to provide benzyl ester **55** is surprisingly faster than transesterification with the α-nucleophile, CbzNHOH.

Table 4. Attempted transesterification of hydroxy methylester **53** with CbzNHOH.



Entry	Conditions	Outcome
1	CbzNHOH, Otera's cat., Tol., reflux	Complex mixture
2	CbzNHOH, Ti(O-*i*Pr)4, DME, 100° C	No Rxn
3	CbzNHOH, I_2, Tol., reflux	No Rxn
4	CbzNHOH, Otera's cat., Tol., reflux	Benzyl ester (**55**)

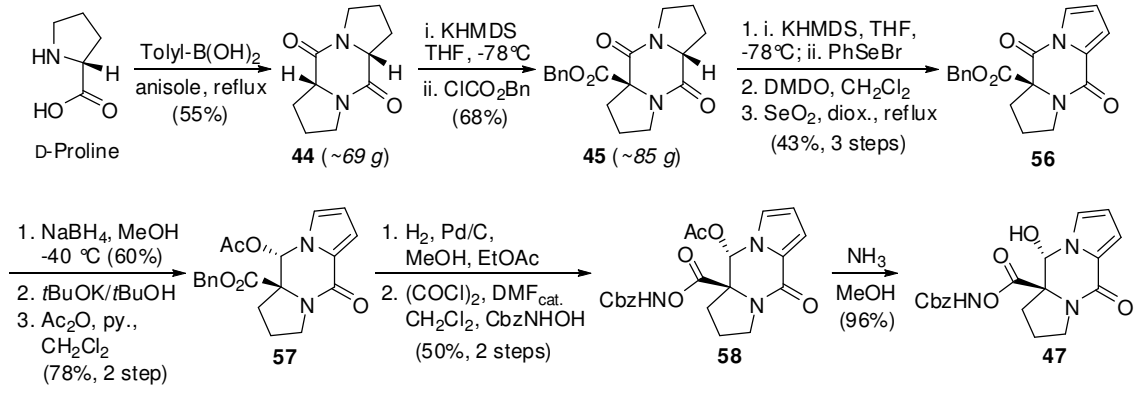
We also tried the transesterification conditions with the *syn* diastereomer **51**, but no desired product was obtained, instead epimerization of the C6 center or no reaction was observed.

B. Revisiting the Original Strategy

Our approach to improve the current methodology encountered several unanticipated problems. Therefore, it was decided to take advantage of the strategy

previously devised in our laboratories for the total synthesis of (+)-dibromophakellstatin and utilize this for the synthesis of the naturally occurring enantiomer (-)-dibromophakellstatin. Employing the original strategy, we wanted to prepare ample quantities of the compounds of interest (*vide supra*) bromophakellstatin, C6-hydroxy phakellstatin along with dibromophakellstatin and phakellstatin for mode of action studies. In order for us to prepare enough material of the desired compounds, we started with bulk amounts of D-proline. We were able to prepare 68.8 g of the C_2-symmetric (-)-cyclo(Pro,Pro) DKP-**44**. Desymmetrization of the DKP under conditions developed in our laboratories for these purposes gave us 84.6 g of the Cbz-DKP **45** (Scheme 17).

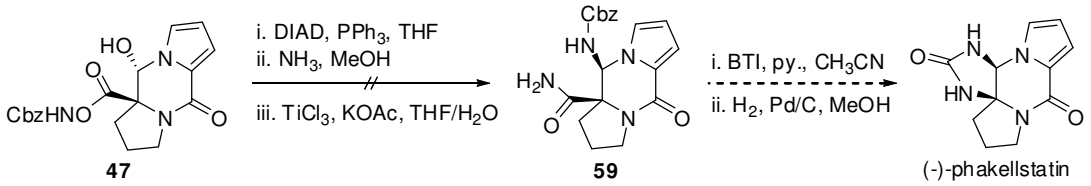
Scheme 17



We proceeded with the synthetic sequence to reach carbinolamine **47**. This was an important intermediate since it would diverge to two synthetic pathways which lead to different compounds of interest (Figure 10, *vide supra*).

With the carbinolamine **47** in hand, we submitted it to the next set of known conditions which entail a three step/one pot reaction to form the β-amino amide **59**. This process invokes an intramolecular delivery of the nitrogen to the C6-position by means of the hydroxamate *via* Mitsunobu conditions (DIAD, PPh_3) in refluxing THF, followed by aminolysis, and cleavage of the N-O bond with titanium trichloride. However, several attempts to reproduce these results were unsuccessful for obtaining the desired β-amine amide **59**.

Scheme 18

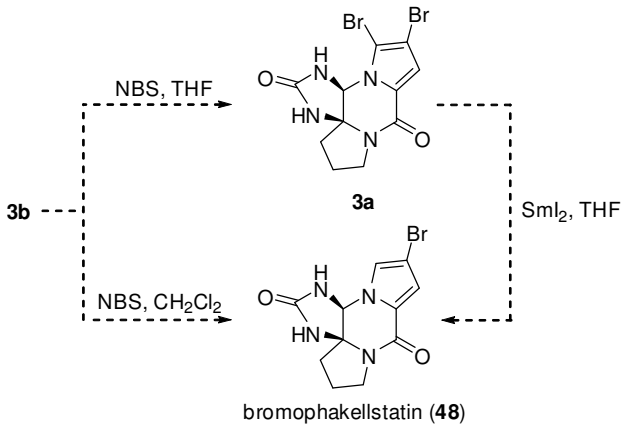


Careful analysis of the reaction conditions and varying reaction times, temperature, and order of addition of reagents did not lead to positive results. Currently, we do no have a concrete explanation for why this three step/one pot sequence did not work in our hands. If the reaction had proceeded to deliver β-amino amide **59** the next step was to submit it to Hofmann rearrangement conditions, namely [bis-(trifluoroacetoxy)]iodobenzene (BTI) in pyridine and acetonitrile, followed by hydrogenolysis of the Cbz group to afford (-)-phakellstatin.[39]

C. Future Studies

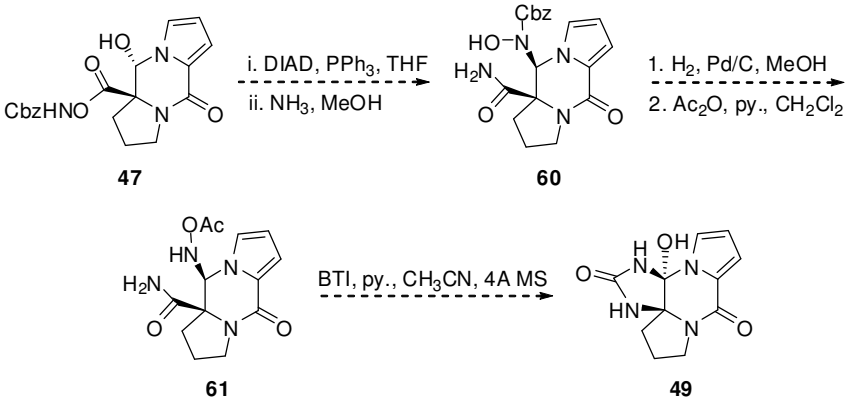
As mentioned before, the material obtained up to the carbinolamine stage would be divided and the sequence would bifurcate to two synthetic pathways. The first one will follow the original method toward completion of the synthesis of (-)-dibromophakellstatin, the naturally occurring enantiomer (Scheme 19). We also plan to study the monobromination of (-)-phakellstatin. Preliminary studies in our laboratories showed promising results although the regioselectivity of the bromination was never confirmed.[5] Even though bromophakellstatin has never been isolated from nature, it is likely to be present as related marine natural products containing monobrominated pyrroles are known, as in the case of the phakellins and palau'amines. Alternatively, if the monobromination fails, a selective reduction by means of SmI_2 could be utilized as has been previously shown by Lindel.[33]

Scheme 19



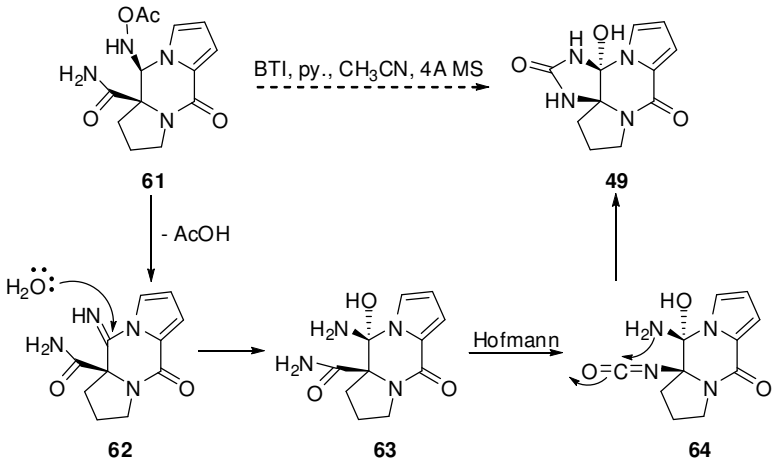
The second pathway will enable further study of the potentially interesting by-product isolated by Dr. K. Poullennec and tentatively assigned as C6-hydroxy phakellstatin (**49**).[5] The latter was found after attempts to introduce the first nitrogen of the urea moiety into the C6 aminal center *via* the Mitsunobu protocol which led to the β-amino amide **60** (Scheme 20). To unmask the β-amine functionality, the benzyloxy carbonyl group was removed by hydrogenolysis. The free hydroxyl amine was subsequently acetylated to give *N*-acetoxy amine **61**. Exposure of **61** to BTI afforded a polar product which was tentatively as **49**.

Scheme 20



The proposed mechanism for this transformation begins with loss of acetic acid *via* E_2 elimination caused by pyridine. Direct oxidation of the β-imino amide **62** can lead to a Hofmann-type rearrangement mediated by the hypervalent iodine species and isocyanate **64** would be trapped by the pendant amino group of the carbinolamine formed by addition of water to the imine moiety (Scheme 21).

Scheme 21



D. Conclusion

Our attempt to streamline the original methodology to (+)-dibromophakellstatin and shorten the synthesis by 5 steps was not fruitful. This forced us to reconsider the original strategy and use it to synthesize (-)-dibromophakellstatin, the natural occurring enantiomer. The sequence was reproducible until we reached carbinolamine **47**. The next step, involving the 3 step/one pot sequence proved problematic and in our hands the reaction was never successful. This precluded us from finishing the synthesis of the natural enantiomer and additional compounds of interest.

CHAPTER III

STUDIES TOWARD (+)-DIBROMOPHAKELLIN AND CONGENERS: A C-H INSERTION STRATEGY

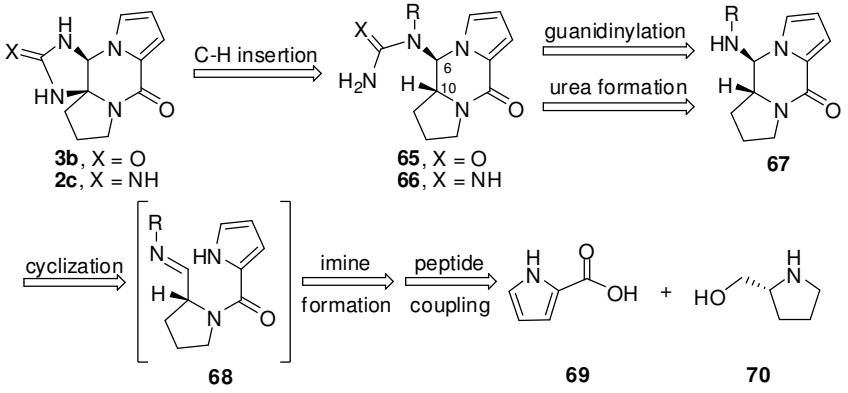
A. Introduction

Previous work from our laboratories which culminated in the synthesis of (+)-dibromophakellstatin provided the first enantioselective approach toward this tetracyclic oroidin class of marine metabolites and it could potentially be applied to the late stages of palau'amine synthesis. However, we recognized that a more concise phakellin annulation strategy would greatly simplify our strategy toward the highly complex alkaloid palau'amine. The second generation strategy towards phakellstatin and phakellin was premised on a C-H insertion process developed by Du Bois and co-workers.[41]

In our first strategy en route for (+)-dibromophakellstatin, we discovered the extreme difference in reactivity/stability of the diamino ketal (C10, phakellstatin numbering) in comparison with the pyrrolo aminal (C6) (see Chapter I, Scheme 8). The lability of the quaternary C10 center is likely due to acyliminium chemistry.[42] Concurrent with our studies, the unusual stability of pyrrole carbinolamines was also observed by Lindel[28] and Evans.[43] Based on the finding that the quaternary C10 amino ketal center of the natural product must be introduced at a late stage of the synthesis due to its instability, the urea/guanidine **65/66** (respectively) was considered a viable precursor of (-)-phakellstatin and (-)-phakellin *via* the Du Bois C-H insertion

methodology (Scheme 22). In principle, **65** and/or **66** could be obtained from hemi-aminal **67** which could in turn arise from cyclization of a transient imine. Final disconnection of the corresponding masked aldehyde leads to the two commercially available substrates, 2-pyrrole carboxylic acid (**69**) and prolinol (**70**).

Scheme 22



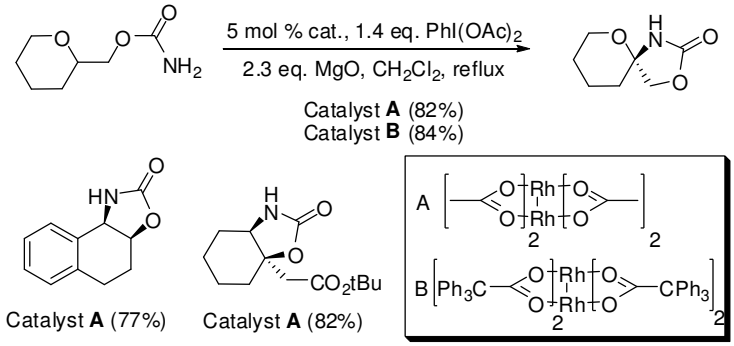
Three main advantages are derived from this strategy. First, it would potentially grant access to both natural products in a unified approach from a common intermediate (**67**). Second, it would reduce the number of steps in the current methodology by more than half (from 16 to 7, longest linear sequence). And lastly, it would expand the scope of the C-H insertion process and the array of possible substrates since the number of natural products that contain either cyclic ureas and/or guanidines is quite vast and this methodology could potentially be used to access some these complex molecules.

1. C-H Insertion with Metallonitrenes

The key step in our second generation approach is a rhodium-catalyzed C-H insertion into the tertiary aminal (C10) center of intermediates **65/66**. Seminal work in the area by Du Bois inspired us to examine this methodology as a mean to rapidly construct the phakellins and phakellstatins.

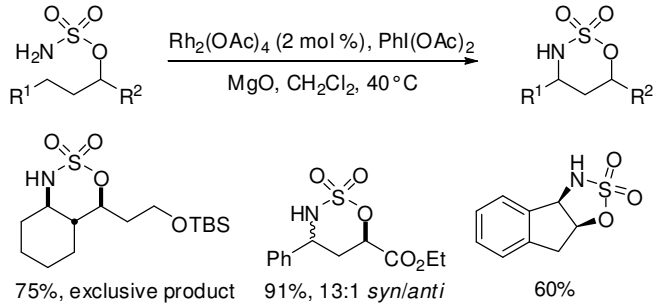
A unique catalytic method for the oxidative cyclization of carbamates to oxazolidinones using [$Rh_2(OAc)_4$] was described in 2001 by Du Bois and co-workers. In this initial account, the authors report that substrates containing both benzylic and tertiary C-H centers underwent cyclization to oxazolidinone products in yields ranging from 74 – 84% (Scheme 23).[41] This reaction is thought to occur through an intramolecular metal-nitrene insertion, though little mechanistic data has been offered in support of this claim.[44] Assertions that direct insertion of a nitrene or nitrenoid intermediate into a methine center should occur with retention of configuration have been posited previously in the literature,[45] for example Meth-Cohn's work pertaining to C-H insertion of nitrenoformates.

Scheme 23



Later that same year, Du Bois and co-workers showed that this methodology could also be used with sulfamate esters to afford oxathiazinanes.[41b] This class of compounds produced exclusive γ-C-H bond amination; finding that contrasted distinctively with their previous results with carbamates (Scheme 24). The authors proposed that the strong bias for oxathiazinanes formation is presumably accounted for by the elongated S-O and the shortened S-N bond (1.58 Å, average length found for both bonds) and the obtuse N-S-O angle (103°) of the sulfamate.[46] It was also noted by the authors that it is possible to efficiently prepare the five-membered sulfamidate in systems where no other alternative cyclization pathway is available. Good yields were obtained (60-91%) and preference for the 1,3-*syn* diastereomers ranged from 4:1 to >20:1.

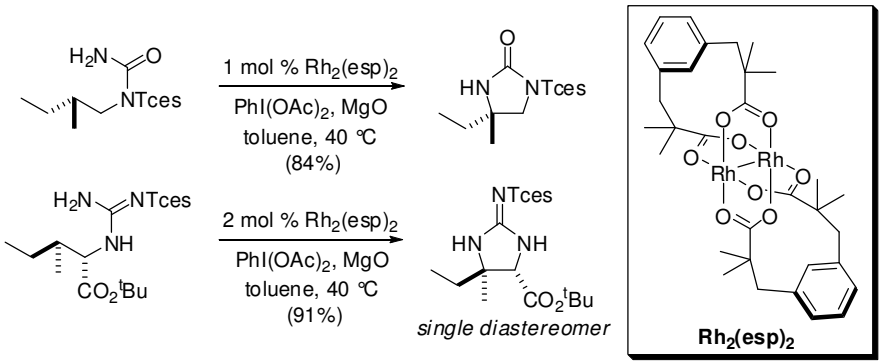
Scheme 24



Importantly, this type of C-H insertion had not been reported employing ureas and/or guanidines moieties as reacting functionalities until fairly recently. Rather, reports to date by Rojas,[47] Padwa,[48] Parker,[49] and others[50] working on the further

development of this C-H amination methodology have employed carbamates and sulfamates. However, Du Bois recently and highly pertinent to our strategy, reported the use of guanidines and ureas for C-H insertion chemistry (Scheme 25).[51] This adds further to the versatility of such processes by increasing its application in synthesis because of its efficiency, predictable selectivity, and the value of the heterocycles that can be produced. For example, heterocyclic ureas and guanidines are found as structural elements in a growing number of complex, biologically efficacious targets, including the greatly diverse family of bromopyrrole alkaloids.

Scheme 25

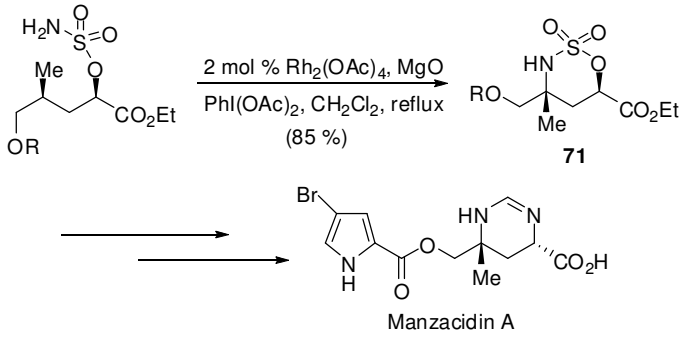


The success of these reactions is predicated on the choice of the electron-withdrawing 2,2,2-trichloroethoxysulfonyl (Tces) protecting group, the commercial catalyst $Rh_2(esp)_2$ (esp = α,α,α',α'-tetramethyl-1,3-benzenedipropionate), and toluene as solvent. When compared to trifluoroacetyl, *p*-tolylsulfonyl, and nosyl, only the Tces group led to an effective oxidative cyclization for both moieties. This group also serves as a useful, robust protecting group for the polar urea and guanidine functionalities.

2. Application to Natural Product Synthesis

The Rh-catalyzed C-H insertion methodology is practical and tolerant of many different functional groups; therefore it is not surprising that this process has been elegantly exploited in natural product synthesis. In their synthesis of the bromopyrrole alkaloids manzacidin A and C in 2002, Wehn and Du Bois used the sulfamate ester-based nitrenoid C-H insertion to form a pivotal C-N bond in **71** in a stereospecific fashion (Scheme 26).[52] By employing this chemistry a rapid, enantioselective synthesis was achieved. The synthesis of manzacidins A and C demonstrated for the first time the efficacy of Rh-catalyzed sulfamate ester insertion reactions in the context of complex natural product synthesis.

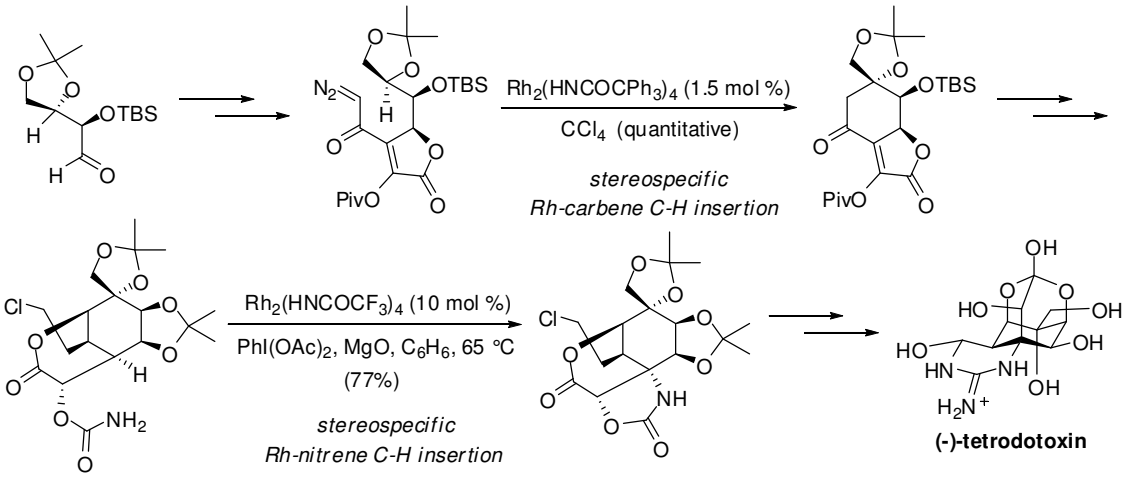
Scheme 26



The carbamate variant of this chemistry was showcased in the synthesis of the potent ion channel blocker, (-)-tetrodotoxin (TTX).[53] This metabolite is one of nature's most marvelous compounds. Its elaborate chemical architecture includes a densely oxygenated cyclohexane framework, a unique ortho-acid, and guanidine functionalities.

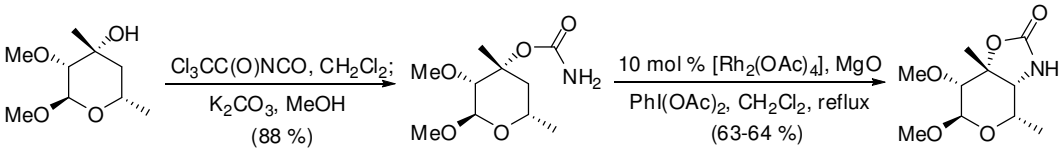
Hinman and Du Bois envisioned in their planning that stereospecific metal-mediated carbene and nitrene C-H insertion reactions would hallmark the defining bond constructions in their approach to TTX (Scheme 27). The utility of the C-H amination is especially noteworthy given the structural complexity of the substrate.

Scheme 27



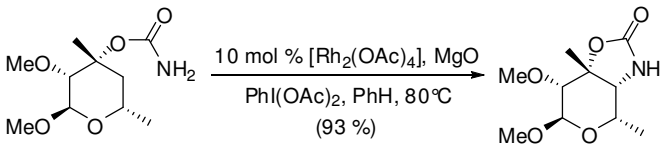
In 2002, Trost and co-workers published the total synthesis of the novel antitumor agent callipeltoside A, and several analogs.[54] The northern part of this molecule is a sugar derived bicycle named methyl callipeltose. The authors used the Du Bois C-H insertion methodology to make the oxazolidinone moiety of this molecule (Scheme 28). With this approach they were able to simplify the synthesis of callipeltose compared to what had been previously reported, leading to the shortest reported synthesis of this sugar moiety.

Scheme 28



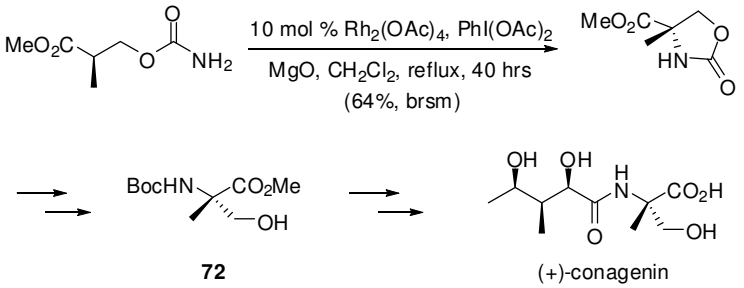
Later, Huang and Panek reported a new approach to an enantioselective synthesis of methyl-L-callipeltose.[55] The final stage of the synthesis was based on Trost's results for the Rh-catalyzed C-H insertion of the carbamate moiety. While Trost and co-workers obtained from 63 to 64% conversion of the C-H insertion product using 10 mol % of the Rh catalyst in refluxing CH_2Cl_2; Panek's laboratory obtained complete conversion using 20 mol % of the catalyst in refluxing CH_2Cl_2. Moreover, they were able to obtain 93% conversion using 10 mol % of the Rh catalyst in refluxing benzene (Scheme 29).

Scheme 29



Recently, a stereoselective synthesis of the immunomodulator (+)-conagenin was described by Yakura and co-workers.[56] The Rh-catalyzed C-H amination reaction was utilized as a key step to synthesize the α-methylserine fragment (**72**) of conagenin (Scheme 30).

Scheme 30



The successful completion of these syntheses offers testament to the power of C-H functionalization as a unique strategy for assembling complex targets and further validates the Rh-catalyzed nitrene insertion as a powerful new tool in synthesis.

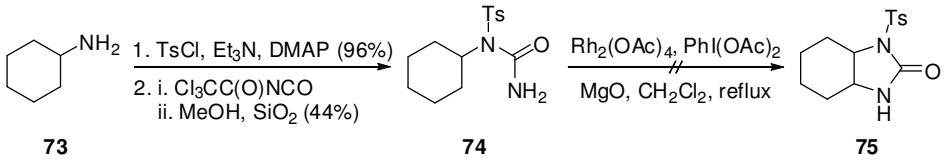
B. Development of a C-H Insertion Strategy

Du Bois and others have reported the application of the oxidative C-H insertion reaction with carbamates and sulfamate esters to produce oxazolidinones and oxathiazinanes respectively. However, when our laboratories initiated studies toward applying this methodology to the phakellins and phakellstatins, it had not been extended to ureas or guanidines. Thus, we planned to probe the viability of this second generation approach utilizing a C-H insertion as a key bond disconnection.

1. Model Studies

Two model systems were designed to initially investigate the aforementioned protocol. In the first model, cyclohexylamine **73** was readily converted to urea **74** by tosylation and consecutive acylation (Scheme 31).

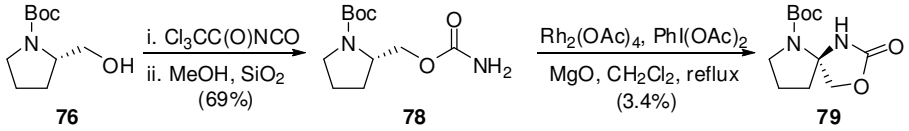
Scheme 31



73 **74** **75**

Disappointingly, exposure of urea **74** to Du Bois C-H insertion conditions did not succeed in delivering bicyclic imidazolidinone **75**. Not discouraged by this result, we next designed a model substrate that would prove more reactive towards C-H insertion, namely a molecule with a methine hydrogen adjacent to a heteroatom. Carbamate **78** posed itself as a suitable model for the construction of a five-membered oxazolidinone. Its synthesis was achieved by acylation of readily available Boc-L-prolinol (**76**) as shown in Scheme 32. Pleasingly, upon exposure to C-H insertion conditions, carbamate **78** delivered spiro-oxazolidinone **79**, albeit in low yield, along with unreacted material.

Scheme 32



76 **78** **79**

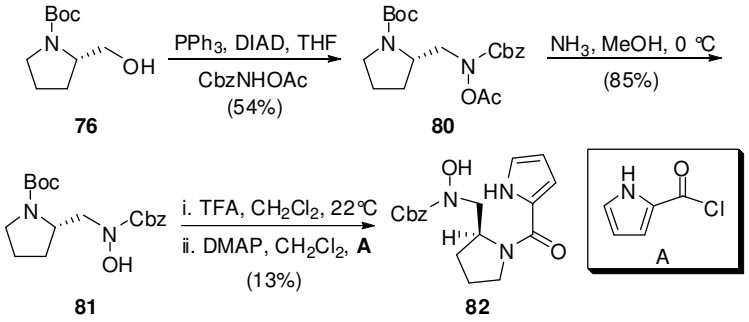
2. Synthesis of the Imine Precursor

To attain the naturally occurring enantiomer of phakellstatin, we would have to begin the synthesis with D-prolinol (**70**). However, we initiated the studies with less

expensive L-prolinol. In the case of dibromophakellin, this is not an important issue since both enantiomers are naturally occurring.[9a, 14]

Our initial synthetic strategy toward the C-H insertion substrate is based on the cyclization of a latent imine. *N*-hydroxy carbamate **82** presented itself as an apposite imine precursor. The synthesis began with Boc-L-prolinol (**76**) and reaction under Mitsunobu conditions with *N*-acetoxy benzylcarbamate (OAcNHCbz) afforded *N*-acetoxy prolinamide **80** in moderate yield (Scheme 33). Isolation of *N*-acetoxy prolinamide **80** proved to be troublesome with yields ranging from 13 to 54%. Following removal of the acetate and Boc protecting groups, the resulting free amine was acylated with pyrrole-2-carbonyl chloride (**A**) to provide *N*-hydroxy carbamate **82** in low yield.

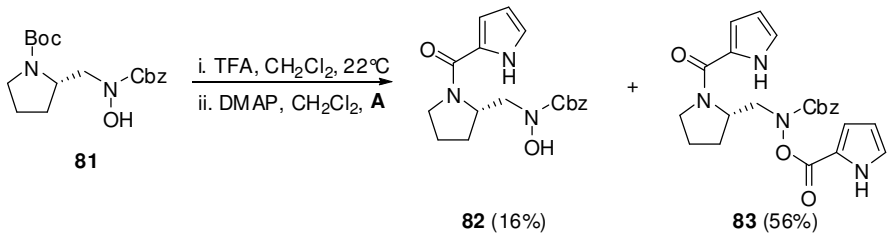
Scheme 33



Attempts to improve the *N*-acylation reaction of *N*-hydroxy prolinamide **81** proved to be a difficult task. After taking a closer look at the reaction, we were able to identify why the reaction was low yielding as a mixture of desired *N*-hydroxy carbamate

82 and bis-acylated carbamate **83** was obtained under reaction conditions with the latter as the major product (Scheme 34). This was not completely unexpected considering the fact that a hydroxyl amine is more nucleophilic than a secondary alkyl amine.

Scheme 34



We tried to hydrolyze bis-acylated carbamate **83** to desired *N*-hydroxy carbamate **82** by treatment with potassium carbonate in methanol, no desired product was obtained. Bis-acylated carbamate **83** proved to be quite robust when exposed to high temperature conditions, namely THF/reflux and toluene 110° C, without undergoing decomposition or any other alteration as judged by TLC and ^{1}H NMR.

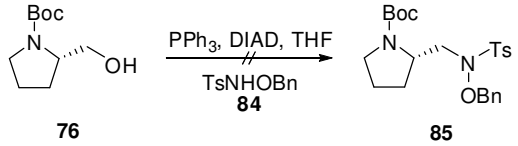
Ideally, subsequent treatment of *N*-hydroxy carbamate **82** to dehydration conditions (TFAA/py.,[57] $ClCO_2Me/Et_3N$,[58] TsCl/DMAP,[59] or Tf_2O/2,6-lutidine[60]) would generate a transient imine, which would cyclize to the enantiomer of hemi-aminal **67** (Scheme 22, *vide supra*).

3. Alternate Approach to the Imine Precursor

Since the Mitsunobu reaction of Boc-L-prolinol **76** with OAcNHCbz did not give good yields and was hard to purify, we decided to change the nucleophile counterpart

expecting better results. When *N*-benzyloxy tosylamine **84** was reacted under Mitsunobu conditions with **76** no desired product was obtained (Scheme 35).

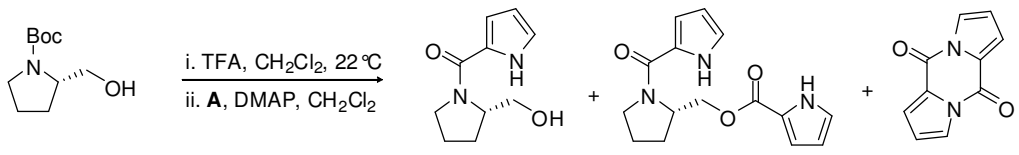
Scheme 35



76 **85**

Efforts to improve the Mitsunobu reaction and nitrogen acylation steps proved challenging so we decided to take an alternate route. We sought to form the annulation precursor (imine precursor) by acylating the proline nitrogen first and then couple this fragment with the secondary amine. Following removal of the Boc protecting group, the free amine was treated with a solution of freshly prepared pyrrole-2-carbonyl chloride (**A**). As noted by TLC, two major spots were identified. After purification we were able to identify these products as *N*-acylated prolinol **86** and bis-acylated prolinol **87** (Scheme 36). Unfortunately, the desired product **86** eluted as an inseparable mixture with pyrrole dimer **88**.

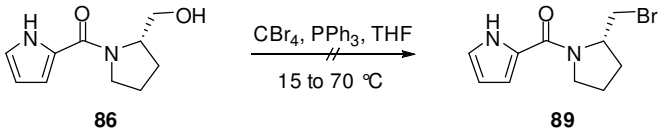
Scheme 36



76 **86** **87** **88**

As another route to couple *N*-acylated prolinol **86** with the secondary amine, we sought to convert the hydroxyl group in **86** to a halogen expecting that a direct S_N2 displacement would be more feasible and less problematic than a Mitsunobu reaction (Scheme 37).[61] Unfortunately, several attempts to replace the hydroxyl group under standard conditions (e.g. CBr_4/PPh_3 and variants) did not lead to the desired bromide **89**.

Scheme 37



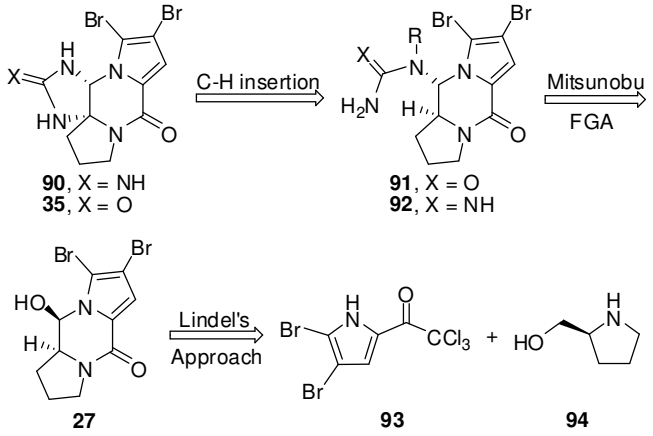
86 **89**

C. Revised Second Generation Approach

Initially it was hoped that the nitrogen at the C6 position would be installed *via* an intramolecular cyclization of the transient imine from *N*-hydroxy carbamate **82**. Alas, all the previous attempts to get to **67** were unproductive. Then, we envisaged using Lindel's approach for the synthesis of the dipyrrolopyrazinone core of the phakellin-like pyrrole-imidazole alkaloids.[28] The *N*,*O*-hemiacetal **27** presented itself as a viable common precursor for both (+)-dibromophakellstatin and (+)-dibromophakellin (Scheme 38). The revised second generation approach stems from previous findings; the lability of the quaternary aminal center C10 (phakellstatin numbering) requires it to be introduce at late stage but the tertiary aminal center C6 is itself stable to various conditions hence it could installed from *N*,*O*-hemiacetal **27**. In principle, the pendant guanidine (**92**) and/or urea (**91**) could be obtained from *N*,*O*-hemiacetal **27** by means of Mitsunobu

reaction/functional group addition (FGA). Final disconnection of the corresponding masked aldehyde leads to dibromopyrrolyl trichloromethylketone **93** and L-prolinol **94**.

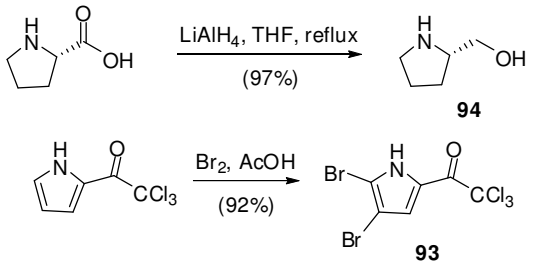
Scheme 38



1. Preparation of N,O-hemiacetal

The preparation of the starting materials L-prolinol[62] **94** and dibromopyrrolyl trichloromethylketone[63] **93** was done following literature procedures and both were obtained in excellent yields (Scheme 39).

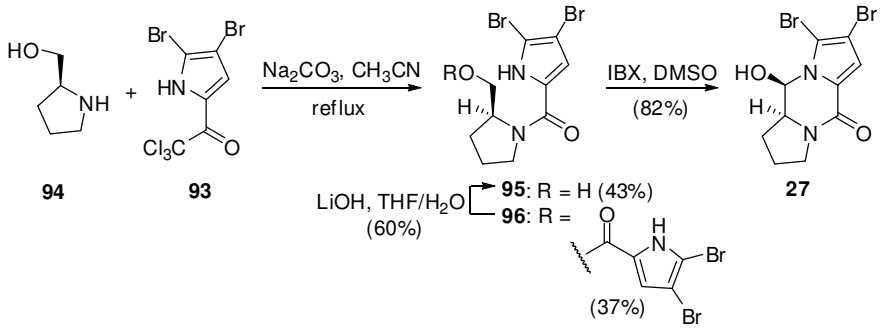
Scheme 39



Once again, to achieve the natural enantiomer of dibromophakellstatin, we would have to begin with D-prolinol (**70**). However, we initiated synthetic studies with the less expensive L-prolinol (**94**). Due to the fact that both enantiomers have been isolated from nature, this is not an issue in the case of dibromophakellin.

The synthesis of the key *N*,*O*-hemiacetal intermediate (**27**) was achieved by peptide coupling of L-prolinol and dibromopyrrolyl trichloromethylketone followed by cyclization using IBX/DMSO (Scheme 40).[28] The peptide coupling reaction gives bis-acylated peptide **96**, as by product, which can be hydrolyzed under saponification conditions to **95** in moderate yield.

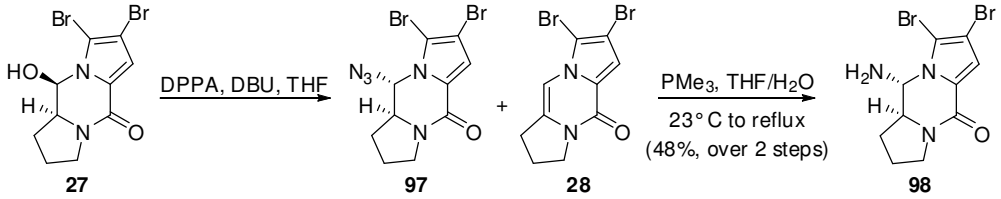
Scheme 40



2. Installation of the Hemi-aminal

Introduction of the first nitrogen of the cyclic urea and/or guanidine onto the C6 position of the ABC core structure of the phakellins and phakellstatins was achieved by treatment of the *N*,*O*-hemiacetal with diphenylphosphoryl azide (DPPA) and DBU (Scheme 41). Attempts to isolate the azide in pure form by silica gel chromatography have failed since one of the by-products of the reaction, enamide **28**, co-elutes with the azide. The mixture was taken onto the next step. We expected to be able to separate hemi-aminal **98** from the eliminated product after reduction, but when the mixture was treated with PPh_3/THF/H_2O the desired product was obtained along with $P(O)Ph_3$ even after purification on silica gel chromatography. Due to the problem of contamination with $P(O)Ph_3$, we decided to find alternative ways to get around this. We did not want to use hydrogenation conditions to reduce the azide because the bromines on the pyrrole ring were prone to cleavage under this condition. A smaller trialkyl phosphine was the reagent of choice since the phosphine oxide by-product is volatile and easily removed under high vacuum conditions.

Scheme 41



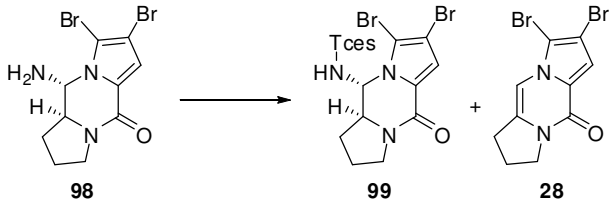
27 **97** **28** **98**

3. N-Activating Group Installation

During the initial studies by the Du Bois group while developing carbamate and sulfamate ester insertion reactions, they discovered that an electron-withdrawing group aids the stability/reactivity of the nitrene intermediate. The nitrene intermediate is believed to be the reactive species that undergoes the oxidative insertion onto the C-H bond with the assistance of a rhodium catalyst. For the *N*-alkyl-*N*-sulfonyl ureas, only the Tces group proved to be effective for oxidative cyclization.[50b]

Once we obtained the hemi-aminal, the substrate was ready to be protected and acylated to form the pendant urea. The reaction of the hemi-aminal with trichloroethoxysulfonyl chloride[64] (Tces-Cl) has proven to be problematic. The main problem is the formation of enamide **28** which decreases the yield of the desired product (Table 5). Ranging from 2 to 10 equivalents of Tces-Cl in conjunction with different nucleophilic and non-nucleophilic bases and different temperatures yielded unpromising results. The highest yield (18%) was obtained when Tces-Cl and DMAP in pyridine as solvent were used (entry 6). But the reaction produces multiple spots which makes the purification difficult.

Table 5. Attempted Tces protecting group installation.



98 **99** **28**

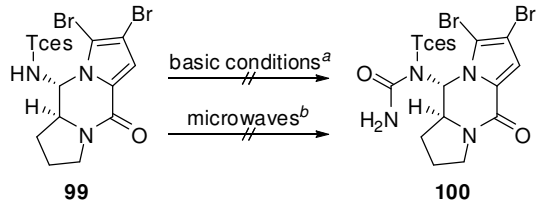
Entry	Conditions	Outcome
1	Tces-Cl (2 eq), Et3N, DMAP, THF, 22° C	**28** + SM
2	Tces-Cl (2 eq), pyridine (2 eq), CH_2Cl_2, 22° C	**28** + SM
3	Tces-Cl (2 eq), pyridine (2 eq), THF, -78° C	**28** + SM
4	Tces-Cl (2 eq), pyridine, 0° C → 22° C	**28** + **99** (16%)[a]
5	Tces-Cl (4 eq), pyridine, DIPEA (4 eq), 0° C → 22° C	**28** + **99** (15%)[a]
6	Tces-Cl (10 eq), DMAP (10 eq), pyridine, 0° C → 22° C	Multiple spots (18%)[a]
7	Tces-Cl (10 eq), PPY (10 eq), pyridine, 0° C → 22° C	Multiple spots (15%)[a]
8	Tces-Cl (5 eq), Et_3N (5 eq), pyridine, 0° → 22° C	**28** + SM

[a] Isolated yield of the product.

4. Acylation of the N-Tces Hemi-aminal

Once with the *N*-Tces protected hemi-aminal in hand we were ready to acylate the nitrogen atom so to form the urea moiety. This difficult process required extensive experimentation that ultimately led to unfruitful results (Scheme 42). Reaction of the hemi-aminal under different basic conditions, varying order of addition of reagents/substrates and temperature, did not yield the desired product. Under microwave forcing conditions no desired pendant urea was observed. Reactivity was observed when utilizing a different isocyanate, trichloroacetyl isocyanate (TAI), but the reaction mixture was complex.

Scheme 42



99 **100**

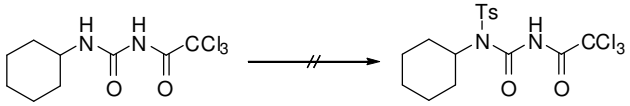
[a] Conditions: Bases like LHMDS, NaH, Cs_2CO_3; TMSNCO as electrophile; from 0° C to reflux in THF. Mostly starting material and some elimination product. [b] TMSNCO and TAI were used; DIPEA was added as acid scavenger; THF as solvent; temperature 110 – 125° C from 30 min. to 1.5 hrs. Some starting material and decomposition.

Using similar reaction conditions we tried to install the pendant urea onto a *N*-tosyl hemi-aminal using TAI, but a complicated mixture of products was obtained.

5. Alternative Approach to Urea Formation

Since efforts to install the protected pendant urea onto the tricyclic core of the phakellstatins have failed, we decided to work on a model system and investigate the reactivity and behavior of these types of systems under variety of reaction conditions. We prepared a model that contained a pendant urea attached to a secondary carbon that was somewhat reminiscent of the real system. *N*-trichloroacetyl-*N*'-cyclohexylurea **101** was prepared by reaction of cyclohexylamine with TAI in good yield (80%). Attempts to selectively protect the amidine moiety resulted unfruitful (Table 6). In all the cases the starting material was recovered.

Table 6. Attempted selective tosylation of *N*-trichloroacetyl-*N'*-cyclohexylurea **101**.



101 **102**

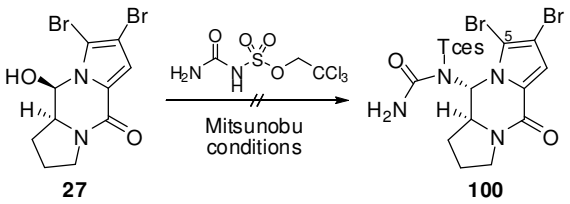
Entry	Conditions
1	TsCl, Et_3N, CH_2Cl_2, 22 to 60 °C
2	TsCl, pyridine, CH_2Cl_2, 22 to 60 °C
3	TsCl, DMAP, CH_2Cl_2, 22 to 60 °C
4	TsCl, Et_3N/DMAP, CH_2Cl_2, 22 to 60 °C
5	Ts_2O, Et_3N/DMAP, CH_2Cl_2, 22 to 60 °C
6	KHMDS (2.1 equiv.), THF, TsCl, 22° C
7	KHMDS (2.1 equiv.), THF, TsCl, 0° C
8	KHMDS (2.1 equiv.), THF, TsCl, -78° C
9	KHMDS (2.1 equiv.), THF, Ts_2O, 0° C
10	KHMDS (2.1 equiv.), THF, Ts_2O, 22° C

6. Installation of the Tces-urea Moiety onto the ABC Core of Dibromophakellstatin

The amidine model system did not serve our purposes as to find ways to install the protected pendant urea. While working on these systems, Du Bois *et al.* published a communication on the preparation of a Tces-protected urea[51b] and its use as nucleophile in Mitsunobu reactions and C-H insertion substrate to construct cyclic ureas.[51] With the Tces-urea in hand we proceeded to try the Mitsunobu reaction to append the urea moiety onto the tricyclic core of the phakellstatins (Scheme 43). Unfortunately, under several reaction conditions varying temperature, time, and the phosphine reagent, either no reaction or just starting material alongside with enamide **28** was observed. It was not

surprising to observe the elimination by-product (**28**) now that, under basic conditions like in the Mitsunobu reaction, dehydration of the *N*,*O*-hemiacetal would be expected. The substrate is set for E_2 elimination leading to a more thermodynamically favored product. The difficulty with the Mitsunobu reaction could be due to the electron density of the bromine in position C5, which might be interfering with the nucleophile and therefore precluding or diminishing the reactivity at the C10 center.

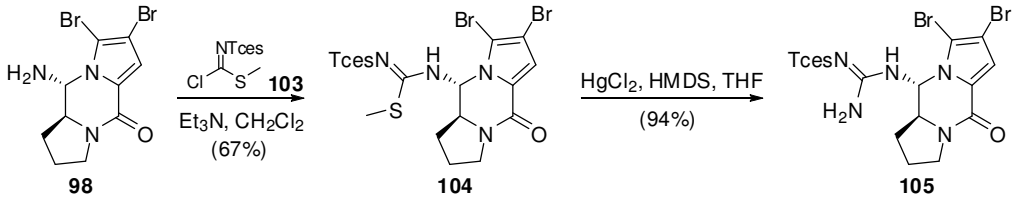
Scheme 43



7. Installation of the Pendant Guanidine Towards Dibromophakellin

Connection of the Tces-urea moiety onto the tricyclic core of the phakellstatins was not a trivial task, so we decided to step back on it for while and focus on the synthesis of dibromophakellin. Formation of the isothiourea intermediate (**104**) was accomplished following a procedure by Saulnier and co-workers.[65] Reaction of the hemi-aminal **98** with imidochloride[51b] **103** proceeded smoothly and in good yield (67%) as shown in Scheme 44. The isothiourea **104** was then successfully converted to the corresponding guanidine in excellent yield (94%).

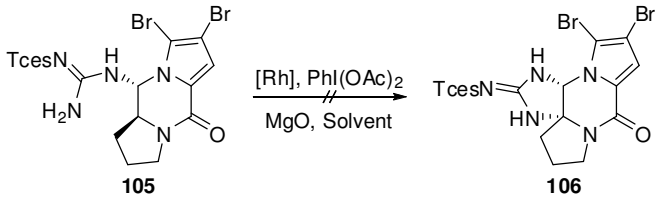
Scheme 44



8. Attempted C-H Insertion

Finally, the much awaited C-H insertion substrate was obtained. Guanidine **105** was submitted to Du Bois C-H amination conditions; disappointingly, it did not yield the desired cyclic guanidine. After increasing catalyst loading (1 – 10 mol %), reaction temperature (40 – 110° C), reaction time (9 – 18 hrs), and changing solvents (toluene to dichloromethane), we hypothesized that the catalyst [$Rh_2(esp)_2$] might be too bulky to actually reach the nitrene intermediate (Scheme 45). We decided to try a smaller dimeric rhodium catalyst that had been used for C-H insertion reaction but with different moieties (i.e. carbamates and sulfamate esters), namely [$Rh_2(OAc)_4$], but this also did not produce the desired product.

Scheme 45



9. Structural Analysis of Aminal and Carbinolamine: C-H Insertion Precursors and Substrates

These results raised the question of the stereochemistry at the guanidine center. We were aware that there may be concerns about regioselectivity of the C-H insertion process, the structure of substrate **105** would appear to preclude the formation of a six-membered cyclic urea as the methylene protons of the pyrrolidine ring are too far away for the insertion process. Furthermore, the desired site of reaction, namely the methine hydrogen at C10, is activated by polarization due to the attached amide nitrogen. Also, direct insertion of the nitrene should occur with retention of configuration premised on the stereospecificity that has been observed by Du Bois and others. We decided to perform nOe studies on our substrates.

a) nOe Studies

Intrigued by the outcome of the C-H insertion reaction, we decided to take a closer look at guanidine **105** and corroborate the relative stereochemistry of both C6 and C10 centers. NOESY 1D experiments were performed on the substrate which revealed that indeed C6 (guanidine-bearing carbon) was inverted and that the pendant guanidine was positioned in the β-face of the molecule in an *anti* relationship to the methine hydrogen at C10 (Figure 11). These positive nOe enhancements provide strong evidence for us to say that the incorrect epimer was obtained.

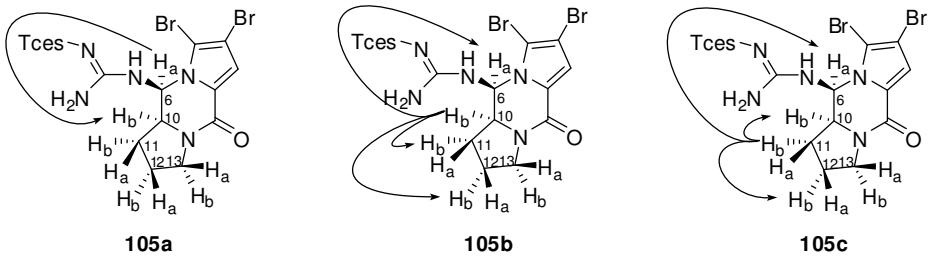


Figure 11. Diagnostic nOe enhancements observed in the guanidine **105**.

b) Coupling Constant Analysis

To supplement the nOe studies, a coupling constant analysis was conducted to support the proposed relative stereochemistry and to verify where in our synthesis the C6 center epimerized. Considering the literature coupling constants (*J*) for *N*,*O*-hemiacetal **26** and **27**,[28] the one obtained experimentally for azide **97** correlated with **26**, meaning that inversion of configuration at that center did occurred. But when we look closely at the coupling constant for hemi-aminal **98** is noticeable that it is now 2.5 Hz, clear indication that it has epimerized and no longer is the amino functional group in the same spatial arrangement as the methine hydrogen at C10; another piece of evidence that supports the inverted relative stereochemistry for C6 relative to C10 (Figure 12).

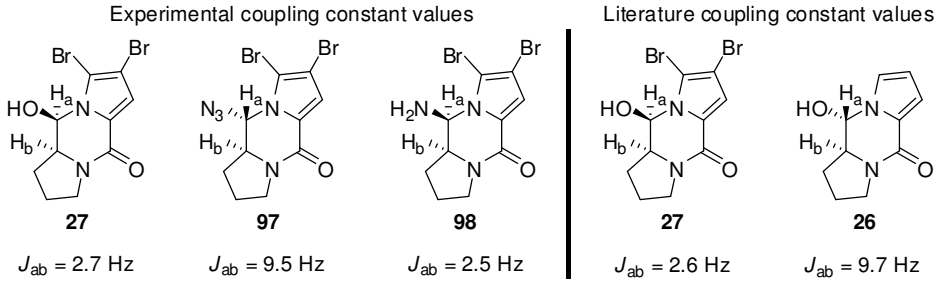


Figure 12. Experimental and literature coupling constants for the intermediates **27**, **97**, and **98**.

c) X-ray Analysis

Fortunately, we were able to do crystallographic structure analysis on the isothiourea **104** which confirmed what the other pieces of evidence were pointing to that the stereochemistry at the C6 position is opposite to what is needed in order for the C-H insertion reaction to proceed. The structure was solved by heavy-atom methods. The ORTEP diagram of one molecule of **104** as viewed along the *y* axis is shown in Figure 13. The coordinates of all atoms found in the electron-density maps, together with their estimated standard deviations (except for hydrogens) are listed in Table 8 of the Appendix B.

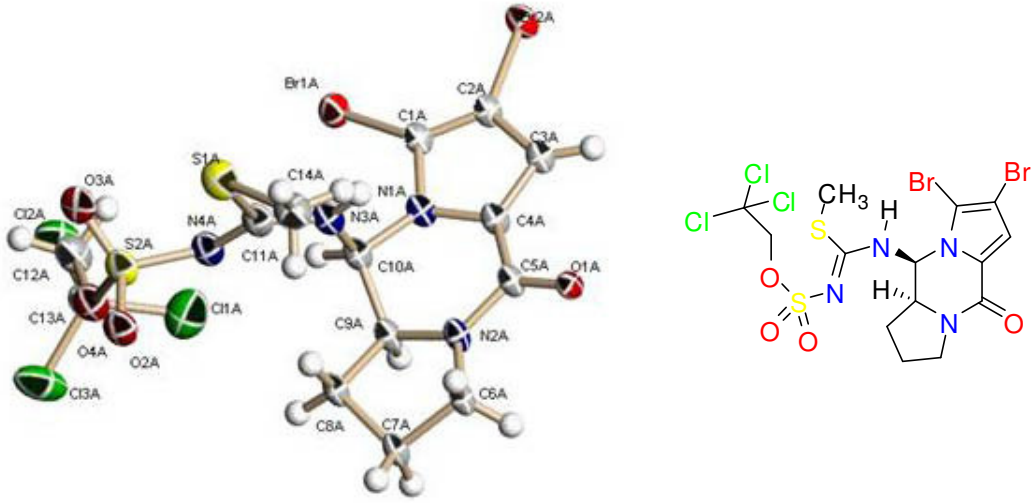


Figure 13. Perspective ORTEP diagram of isothiourea **104**. The thermal vibration ellipsoids are shown on a 50% probability scale.

Crystals suitable for X-ray diffraction were obtained for guanidine **105**. The absolute stereochemistry for the two chiral centers of guanidine **105** were assigned (using X-ray numbering) as 9*S*, 10*S*. The skeleton of guanidine **105** as revealed by X-ray analysis is in complete accord with the one proposed on the basis of spectroscopic data and chemical studies. The crystallographic data indicate that the pendant guanidine moiety is in the *trans*-position relative to the hydrogen at C9 (X-ray numbering). The ORTEP diagram of one molecule of **105** as viewed along the *y* axis is shown in Figure 14.

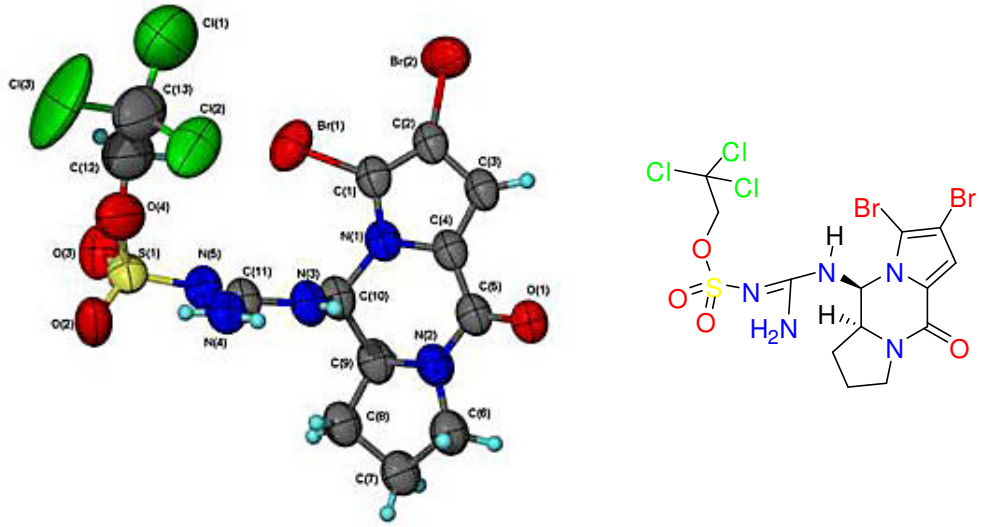
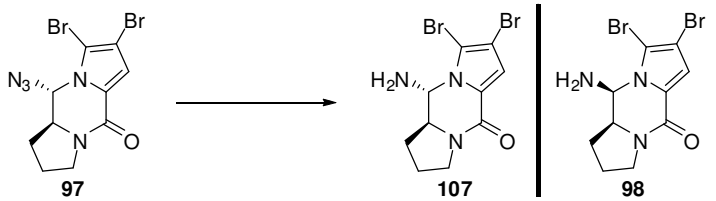


Figure 14. Perspective ORTEP diagram of guanidine **105**. The thermal vibration ellipsoids are shown on a 50% probability scale.

10. Inversion or Retention

The question of stereochemistry at the guanidine bearing carbon has been answered by X-ray structure analysis. The query now is to investigate when epimerization is occurring. According to our coupling constant analysis and further studies on the formation of azide **97**, inversion of configuration at C6 did happen at this stage. After reduction of the azide under variety of conditions, it is clear that epimerization is indeed occurring at this point (Table 7).

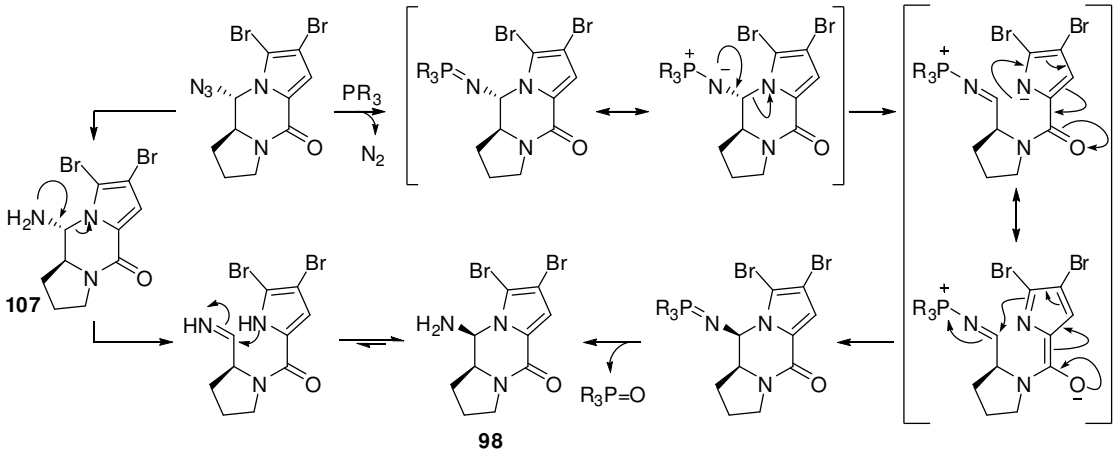
Table 7. Studies on the reduction reaction of azide **97**.



Entry	Conditions	Outcome (107:98)
1	PMe_3, THF, 30 min. 0°C, then H_2O, reflux 1 h	**98**
2	PMe_3, THF, 30 min. 0°C, then H_2O, 22° C, 8 h	**98**
3	PPh_3, THF, 30 min. 22°C, then H_2O, 22° C, 8 h	**98**
4	H_2, Lindar's catalyst, MeOH	N.R.
5	$SnCl_2$, MeOH	**98**
6	SmI_2, THF	Complex mixture

We surmise that epimerization occurs at the stage of iminophosphorane formation. A plausible mechanism by which the epimerization is occurring is outlined below (Scheme 46). Alternatively, epimerization could occur following hydrolysis of the iminophosphorane by simple ring opening to an imine and reclosure (**107** → **98**).

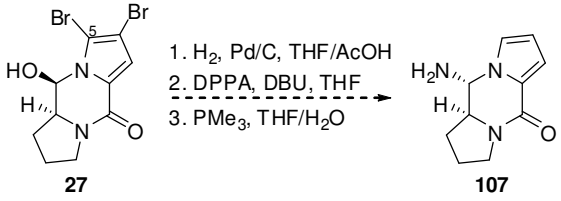
Scheme 46



11. Correction of Stereochemistry

We plan to correct the stereochemistry at the C6 center by removal of the bromines on the pyrrole ring of the *N*,*O*-hemiacetal **27** prior to the installation of the hemi-aminal. As observed previously by Lindel,[28] there appears to be an enthalpic difference that governs the anomeric equilibrium at the carbinolamine C6 center dictated primarily by the C5 substitution. When this position is brominated, the preferred diastereomer possess a β-hydroxy group and when lacking the C5-bromine a product mixture is obtained favoring the α-hydroxy diastereomer in a 9:1 ratio. Cleavage of the bromines by hydrogenolysis and subsequent treatment with DPPA/DBU followed by PMe_3/THF/H_2O should afford the correct hemi-aminal (**107**) as shown in Scheme 47.

Scheme 47



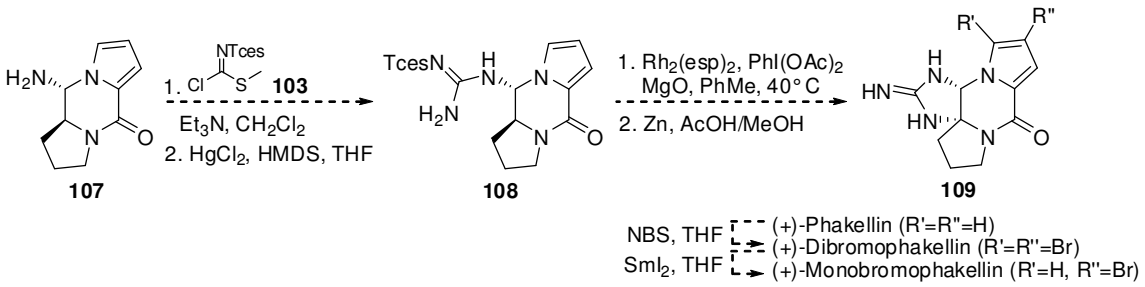
27 **107**

D. Proposed End Game: Completion of the Synthesis

Once the stereochemistry problem at C6 is corrected, installation of the isothiourea by means of the imidochloride **103** previously used followed by treatment with $HgCl_2$/HMDS to deliver the pendant guanidine **108** (Scheme 48). The primary concern for regioselectivity in the C-H insertion process for this substrate would appear to involve the aromatic pyrrole hydrogen at C5. This regioselectivity will need to be

determined experimentally, however if this is found to be problematic, the reaction could be performed following bromination of the pyrrole, which would preclude this reaction pathway.

Scheme 48



107 108 109

E. Conclusion

We devised an alternative approach to the phakellins and phakellstatins based on a Rh-catalyzed C-H amination protocol developed by Du Bois and co-workers. Initial model studies for the pivotal C-H insertion reaction proved promising for this second generation strategy. Though our early second generation approach to construct aminal **67** *via* transient imine **68** resulted on a dead end, we were able to take advantage of Lindel's procedure to synthesize the dipyrrolopyrazinone core (ABC ring system) of the phakellins and phakellstatins. Further functionalization of the *N*,*O*-hemiacetal (**27**) led to the formation of the crucial pendant guanidine **105** for the synthesis of (+)-dibromophakellin, unfortunately this key substrate resulted to be incorrect diastereomer which prohibited us to study the crucial C-H insertion reaction. Ultimately, a similar synthetic approach could be applied to the urea containing alkaloid,

dibromophakellstatin. Importantly, this new expedient approach could be directly applicable to the projected synthesis of palau'amine.

CHAPTER IV

CONCLUSION

In summary, our approach to streamline the strategy developed in our laboratories for the synthesis of (+)-dibromophakellstatin proved to be quite a challenge. Consequently, our ability to reproduce the original approach towards this marine alkaloid was hampered by a problematic Mitsunobu reaction which precluded us from finishing the synthesis of natural enantiomer (**3a**) and additional compounds of interest (see Figure 10).

On the other hand, our second generation approach shows promise as an efficient route to the phakellins and it has potential to be applicable to the phakellstatins. We designed a method that allows us to reach the key C-H insertion precursor in 6 steps (longest linear sequence) from commercially available prolinol (D or L). However, the current route provides the undesired *anti* diastereomer for the key pendant guanidine, which precluded the study of the pivotal C-H insertion process. This approach, if perfected, would grant access to the targeted alkaloids *via* the C-H amination of a urea and/or of a guanidine to introduce the essential quaternary aminal center. This would be a more amenable approach to the annulation of the phakellin substructure in the final stages of palau'amine synthesis. Furthermore, this methodology could potentially be applied to the synthesis of other complex oroidin-derived marine alkaloids.

CHAPTER V

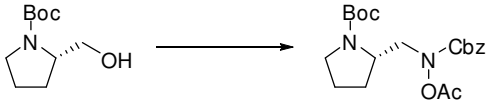
EXPERIMENTAL PROCEDURES

General

All non-aqueous reactions were carried out under a nitrogen atmosphere in oven-dried (130° C) glassware unless noted otherwise. Tetrahydrofuran (THF), diethyl ether (Et_2O), methylene chloride (CH_2Cl_2), acetonitrile (CH_3CN), dimethylformamide (DMF), and toluene (PhMe) were dried and purified by a MBRAUN solvent purification system by passage under 8 psi N_2 through activated molecular sieves. Methanol (MeOH) was distilled from magnesium prior to use. Dimethylsulfoxide (DMSO, 99.7% AcroSeal) was purchased from Acros and used as received. Pyridine (py) was purchased from EM Science and stored under potassium hydroxide (KOH). Triethylamine (Et_3N), diisopropylamine, and diisopropylethylamine (DIPEA) were distilled from calcium hydride prior to use. Dimethyldioxirane (DMDO)[68] in acetone, phenylselenyl bromide, and 2-iodoxybenzoic acid (IBX)[69] were prepared following literature protocols. All other commercially available reagents were used as received. Brine refers to a saturated solution of sodium chloride. Reactions were followed by thin layer chromatography (TLC) on SiliCycle® silica gel 60 Å F_{254} (250 μm). Visualization of the developed plate was accomplished by fluorescence quenching and by staining with ethanolic anisaldehyde, aqueous potassium permanganate ($KMnO_4$), ceric ammonium molybdate (CAM), or vanillin. Chromatographic purification of compounds was accomplished by flash chromatography on SiliCycle® Silia*Flash*® F60, 40-63 μm 60 Å.

Nuclear magnetic resonance (NMR) spectra were acquired on a Varian Inova-500 MHz or Inova-300 MHz operating at 500 and 125 MHz, and 300 and 75 MHz for ^{1}H and ^{13}C, respectively, and are referenced internally according to residual solvent signals. ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz) and multiplicity is abbreviated as follows: *app* = apparent, s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, p = pentet, m = multiplet, *br* = broad signal. IR spectra were acquired using a Bruker Tensor 27 FTIR spectrophotometer. Vibration frequencies are expressed in cm^{-1}. Mass spectra were obtained on a MDS Sciex (Concord, Ontario, Canada) API Qstar Pulsar (for ESI) or a ThermoFinnigan (San Jose, California) LCQ Deca Mass Spectrometer (for APCI) at the Laboratory for Biological Mass Spectrometry (LBMS) at Texas A&M University.

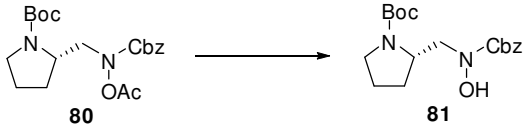
Procedures



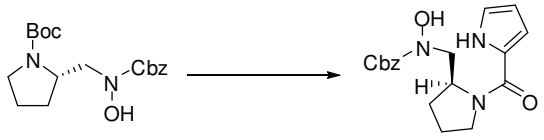
76 **80**

***N*-Acetoxy prolinamide 80.** A solution of Boc-L-prolinol (534.4 mg, 2.655 mmol) and triphenylphosphine (767.2 mg, 2.920 mmol) in THF (10 mL) was treated with DIAD (575.0 μL, 2.920 mmol), followed by CbzNHOAc (666.1 mg, 3.186 mmol). After 30 minutes at 23° C, the volatiles were removed under reduced pressure and the residue was purified by flash column chromatography (SiO_2, hexanes/EtOAc, 9:1) to provide *N*-acetoxy prolinamide **80** (564.2 mg, 54%) as a colorless oil: R_f 0.13 (hexanes/EtOAc, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.40 (m, 5H), 5.18 (s, 2H), 3.88-4.05 (m, 1H),

3.69-3.83 (m, 2H), 3.28-3.40 (m, 2H), 2.14 (s, 3H), 1.76-1.98 (m 4H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.5, 156.3, 135.0, 128.5, 128.3, 128.2, 127.8, 68.16, 55.10, 52.15, 51.42, 46.25, 29.61, 29.30, 22.57, 18.21; IR ($CDCl_3$) 1797, 1729, 1694; HRMS (ESI) calculated for $C_{20}H_{28}N_2O_6$ [M+Na] 415.1845. Found 415.1827.

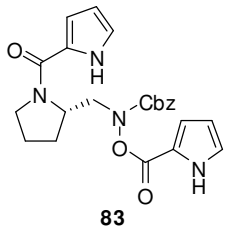


***N*-hydroxy prolinamide 81.** Ammonia (gas) was bubbled into a solution of *N*-acetoxy carbamate (564.0 mg, 1.438 mmol) in MeOH (6 mL) at 0° C for 2 minutes. After stirring the mixture for 15 minutes at 0° C, the volatiles were removed under reduced pressure and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 4:1) to provide *N*-hydroxy prolinamide **81** (428.9 mg, 85%) as a colorless oil; R_f 0.69 (hexanes/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 1H), 7.25-7.33 (m, 5H), 5.20-5.02 (*AB*, *J* = 12.0, 41.7 Hz, 2H), 4.20-4.26 (m, 1H), 3.80 (*app* t, *J* = 24.0 Hz, 1H), 3.15-3.26 (m, 3H), 1.88-1.94 (*br* m, 3H), 1.56-1.60 (m, 1H), 1.39 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6, 156.3, 134.9, 128.4, 128.3, 127.9, 68.25, 67.26, 53.18, 46.38, 28.36, 23.29, 20.89, 18.26; IR ($CDCl_3$) 3232, 2976, 1737, 1690, 1399; LRMS (APCI) calculated for $C_{18}H_{26}N_2O_5$ [M+H] 351.1. Found 351.1.



81 **82**

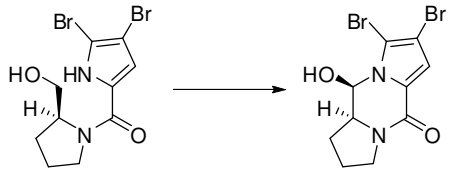
***N*-hydroxy carbamate 82.** To a solution of *N*-hydroxy prolinamide (100.0 mg, 0.2855 mmol) in CH_2Cl_2 (25 ml) was added trifluoroacetic acid (TFA) (198.2 μL, 2.569 mmol). After stirring at 22° C for 22 h, the volatiles were removed *in vacuo*. A freshly prepared solution of pyrrole-2-carbonyl chloride (**A**) was added *via* cannula to a solution of free amine and DMAP (175.0 mg, 1.432 mmol) in CH_2Cl_2 (25 mL). After stirring at 22° C for 7.5 h, the volatiles were removed under reduced pressure and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 7:3) to provide *N*-hydroxy carbamate **82** (12.7 mg, 13%) as a yellowish oil: R_f 0.55 (hexanes/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.48 (s, 1H), 9.39 (s, 1H), 7.20 (*br* s, 3H) 6.96 (*app* s, 1H), 6.64 (*app* s, 1H) 6.31 (*app* t, *J* = 5.4 Hz, 1H) 4.93-5.02 (*AB*, *J* = 12.3, 14.1 Hz, 2H), 4.81 (m, 1H), 3.96 (*app* t, *J* = 14.1 Hz, 1H), 3.76 (*br* s, 2H), 3.31-3.37 (dd, *J* = 3.9, 14.1 Hz, 1H), 2.14 (m, 2H), 2.01 (m, 2H) ; LRMS (ESI) calculated for $C_{18}H_{21}N_3O_4$ [M+H] 344.1. Found 344.1.



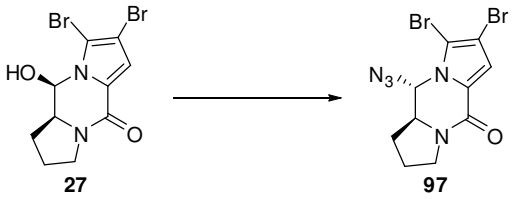
83

Bis-acylated compound 83. R_f 0.32 (hexanes/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (*br*, 1H), 9.63 (*br*, 1H), 7.29 (s, 5H), 6.97-6.86 (m, 3H), 6.57 (*br*, 1H),

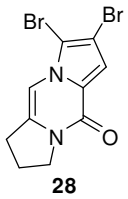
6.21 (*br*, 2H), 5.17 (s, 2H), 4.64 (*br*, 1H), 4.04-4.02 (m, 2H), 3.79 (*br*, 2H), 2.12-1.94 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.94, 158.61, 155.95, 135.55, 128.41, 128.10, 127.73, 125.47, 124.88, 121.57, 118.62, 117.68, 112.73, 110.69, 109.81, 68.20, 56.42, 51.34, 48.61, 27.37, 24.42; IR ($CDCl_3$) 3339, 3252, 1716, 1583, 1440; LRMS (APCI) calculated for $C_{23}H_{24}N_4O_5$ [M+Na] 459.1. Found 459.1.



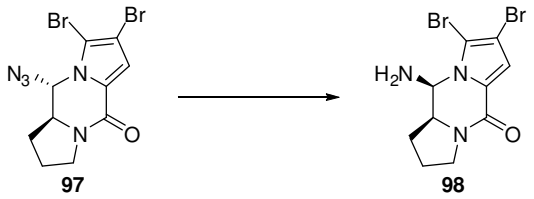
***N,O*-hemiacetal 27.** IBX (333.3 mg, 1.189 mmol) was suspended in DMSO (2.0 mL). After 25 minutes of stirring at 22° C the solution became homogeneous and *N*-acyl prolinol was added (209.4 mg, 0.5948 mmol). The mixture was stirred for 14 h at 22° C. The solution was then diluted with 25 mL of water. The precipitate was filtered off, then washed three times with CH_2Cl_2 (3 x 30 mL). The organic layers were combined, dried under $MgSO_4$, and filtered. Removal of volatiles *in vacuo* gave *N*,*O*-hemiacetal **27** as a white solid (170.1 mg, 82%): R_f 0.47 (EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.05 (d, *J* = 8 Hz, 1H), 6.85 (s, 1H), 5.65 (dd, *J* = 2.5, 8 Hz, 1H), 4.08-4.04 (m, 1H), 3.61-3.57 (m, 1H), 2.13-2.10 (m, 1H), 2.04-1.97 (m, 3H), 1.88-1.85 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.55, 127.34, 114.01, 106.77, 100.92, 76.78, 61.18, 45.22, 27.81, 23.47. Data matched that previously reported.[28]



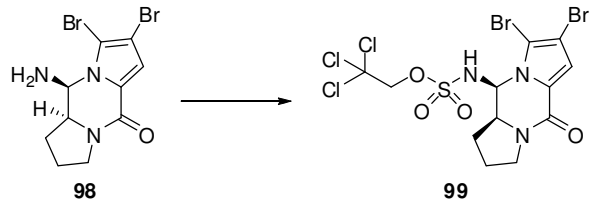
Azide 97. A solution of *N*,*O*-hemiacetal (93 mg, 0.27 mmol) and DBU (64 μL, 0.42 mmol) in THF (10 mL) was treated with DPPA (92 μL, 0.42 mmol) and stirred at 22° C for 15 minutes. The volatiles were removed under vacuum and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 100:0 → 3:1 → 1:1 → 1:2) to provide azide **97** as a yellow solid which was contaminated with enamide **28**: R_f 0.67 (EtOAc); ^{1}H NMR (500 MHz, acetone-d_6) δ 6.89 (s, 1H), 5.83-5.85 (d, J = 9.5 Hz, 1H), 3.99 (m, 1H), 3.64 (m, 1H), 3.50 (m, 1H), 1.92-2.20 (m, 4H); IR (neat, ATR) 2121, 1644, 1440.



Enamide 28. R_f 0.62 (EtOAc); ^{1}H NMR (500 MHz, acetone-d_6) δ 7.20 (dt, J = 3, 3.5 Hz, 1H), 7.04 (d, J = 1 Hz, 1H), 3.96 (t, J = 7 Hz, 2H), 3.07 (dt, J = 1.5, 7.5 Hz, 2H), 2.24 (p, J = 7 Hz, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 153.3, 133.8, 132.7, 130.2, 110.8, 103.1, 101.2, 100.9, 47.50, 23.15; LRMS (APCI) calculated for $C_{10}H_8N_2OBr_2$ 331.9. Found 331.1. Data matched that previously reported.[28]

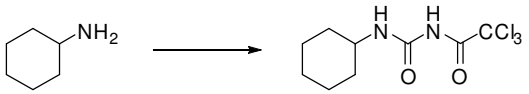


Hemi-aminal 98. A solution of crude azide (93 mg, 0.27 mmol) in THF (3.0 mL) at 0° C was treated with PMe_3 (0.70 mL, 0.66 mmol). The mixture was stirred at 0 °C for 30 minutes. Then, H_2O was added (1 equiv) and the mixture was refluxed for 1 h. The volatiles were removed under vacuum and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 100:0 → 3:1 → 1:1 → 1:2) to get hemi-aminal **98** (44.7 mg, 48%) over 2 steps as an off-white solid: R_f 0.15 (EtOAc); ^{1}H NMR (500 MHz, acetone-d_6) δ 6.74 (s, 1H), 5.25 (d, J = 2.5 Hz, 1H), 4.17 (m, 1H), 3.61 (m, 1H), 3.44 (m, 1H), 2.28 (m, 1H), 2.18 (m, 1H), 1.91 (m, 2H); IR (ATR, neat) 3319, 3278, 1626; LRMS (ESI) calculated for $C_{10}H_{11}Br_2N_3O$ [M+H] 350.0. Found 349.9.



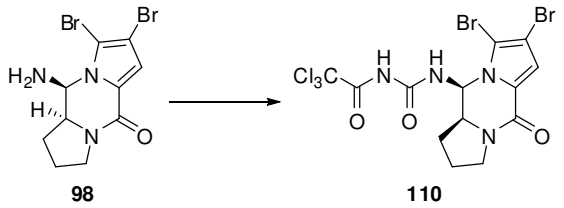
***N*-Tces hemi-aminal 99.** A solution of hemi-aminal (23.5 mg, 0.067 mmol) in pyridine (0.25 mL) at 0 °C was treated with Tces-Cl[64] (33.4 mg, 0.135 mmol) added slowly down the wall of the reaction flask. Solution went from colorless to almost red over several hours. The reaction was allowed to warm-up to 22° C and stirred at 22° C for 6 h. The volatiles were removed under vacuum and the residue was purified by flash

chromatography (SiO_2, hexanes/EtOAc, 100:0 → 5:1 → 4:1 → 3:1 → 2:1 → 1:1 → 1:2) to provide sulfamate **99** (6.2 mg, 16%) as a yellow oil: R_f 0.6 (EtOAc); ^{1}H NMR (500 MHz, acetone-d_6) δ 6.81 (s, 1H), 6.09 (d, *J* = 3 Hz, 1H), 4.49-4.73 (*AB*, *J* = 11, 110 Hz, 2H), 4.44 (m, 1H), 3.62 (m, 1H), 3.46 (m, 1H), 2.58 (d, *J* = 8 Hz, 1H), 2.38 (m, 2H), 2.18 (m, 2H); LRMS (ESI) calculated for $C_{12}H_{12}Br_2Cl_3N_3O_4S$ [M+H] 561.5. Found 561.8.



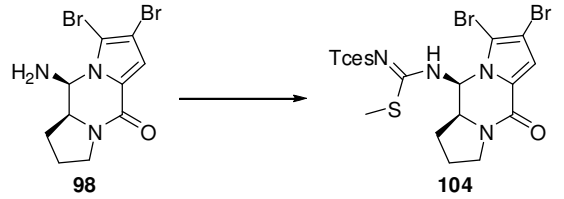
101

***N*-trichloroacetyl-*N'*-cyclohexylurea 101.** A solution of cyclohexylamine (115 μL, 1.00 mmol) in THF (5 mL) was treated with TAI (0.59 mL, 5.02 mmol). The mixture was stirred at 22° C for 3 h. The volatiles were removed under vacuum and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 10:1) to provide *N*-trichloroacetyl-*N'*-cyclohexylurea **101** as a white solid (230.6 mg, 80%): R_f 0.5 (hexanes/EtOAc, 6:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.81 (d, *J* = 6 Hz, 1H), 3.72-3.78 (m, 1H), 1.96 (m, 2H), 1.73 (m, 2H), 1.61 (m, 1H), 1.23-1.44 (m, 5H); IR (ATR, neat) 3352, 3076, 1717, 1696, 1537, 1495; LRMS (APCI) calculated for $C_9H_{13}Cl_3N_2O_2$ 287.6. Found 286.9.



98 **110**

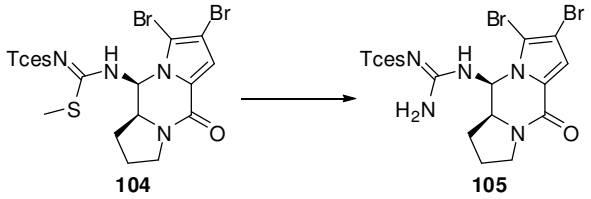
Imidine 110. A solution of hemi-aminal **98** (33.4 mg, 0.096 mmol), azeotroped with xylenes, in THF (0.5 mL) was treated with TAI (56.7 μL, 0.478 mmol). The mixture was stirred at 22° C overnight. The volatiles were removed under vacuum and the residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 1:1) to provide imidine **110** as a tan color solid (38.3 mg, 75%): R_f 0.6 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.34 (d, *J* = 9 Hz, 1H), 6.93 (s, 1H), 6.38-6.43 (dd, *J* = 3.3, 9.6 Hz, 1H), 4.22 (m, 1H), 3.78 (m, 1H), 3.58 (m, 1H), 2.29 (m, 1H), 2.12 (m, 1H), 1.82-2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.7, 156.3, 150.4, 125.9, 116.4, 107.0, 102.8, 60.80, 60.37, 44.69, 28.04, 23.28; IR (CH_2Cl_2) 1779, 1723, 1623, 1519; LRMS (ESI) calculated for $C_{13}H_{11}Br_2Cl_3N_4O_3$ 537.4. Found 537.8.



98 **104**

Isothiourea 104. A pre-cooled solution at 0° C of imidochloride **103**[51b] (34.7 mg, 0.108 mmol) in CH_2Cl_2 (0.3 mL) was treated with a solution of hemi-aminal **98** (34.3 mg, 0.098 mmol) in CH_2Cl_2 (0.5 mL), which was added dropwise followed by Et_3N (16.4 μL, 0.118 mmol). The mixture was allowed to reach 22° C, and then the flask was

immersed in an oil bath at 55° C and stirred overnight. The volatiles were removed under vacuum and the residue was purified by SiO_2 column chromatography (hexanes/EtOAc, 3:1 → 1:1 → 1:2) to provide isothiourea **104** (41.5 mg, 67%) as a tan color solid: R_f 0.65 (EtOAc); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.24 (*br* s, 1H), 6.53 (*br* s, 1H), 6.35 (*br* s, 1H), 4.53-4.52 (*app* s, 2H), 4.11 (*br* s, 1H), 3.36 (m, 1H), 3.11 (m, 1H), 2.28 (s, 3H), 2.05 (m, 1H), 1.80 (m, 1H), 1.56-1.68 (m, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 169.8, 156.1, 128.4, 114.9, 106.9, 102.0, 95.06, 79.68, 79.46, 63.69, 60.62, 44.93, 23.74, 15.28; IR (neat, ATR) 3220, 1717, 1646, 1504, 1444, 1353, 1163; LRMS (ESI) calculated for $C_{14}H_{15}Br_2Cl_3N_4O_4S_2$ [M+H] 634.6. Found 634.8.



Guanidine 105. A solution of isothiourea **104** (21.6 mg, 0.034 mmol) in THF (0.30 mL) at room temperature (22° C) was treated with $HgCl_2$ (10.2 mg, 0.037 mmol) followed by HMDS (18.5 μL, 0.085 mmol). After stirring at 22° C for 5 h the mixture was filtered through Celite™, followed by wash with EtOAc, the volatiles were removed under vacuum. The residue was purified by flash chromatography (SiO_2, hexanes/EtOAc, 100:0 → 3:1 → 1:1 → 1:3) to provide guanidine **105** as a tan solid (19.3 mg, 94%). R_f 0.35 (EtOAc/hexanes, 3:1); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.03 (d, J = 9 Hz, 1H), 6.91 (*br* s, 2H), 6.66 (s, 1H), 6.58 (d, J = 9.5 Hz, 1H), 4.75 (*app* t, J = 13 Hz, 2H), 4.41 (m, 1H), 3.68 (m, 1H), 3.46 (m, 1H), 2.36 (m, 2H), 2.01-1.91 (m, 2

H); ^{13}C NMR (125 MHz, acetone-d_6) δ 157.44, 156.83, 126.58, 115.25, 107.28, 102.25, 95.42, 78.86, 62.29, 61.36, 45.55, 28.34, 23.79; IR (neat, ATR) 3450, 3347, 1725, 1630, 1535, 1444, 1305, 1175; LRMS (ESI) calculated for $C_{13}H_{14}Br_2Cl_3N_5O_4S$ [M+H] 603.5. Found 603.8.

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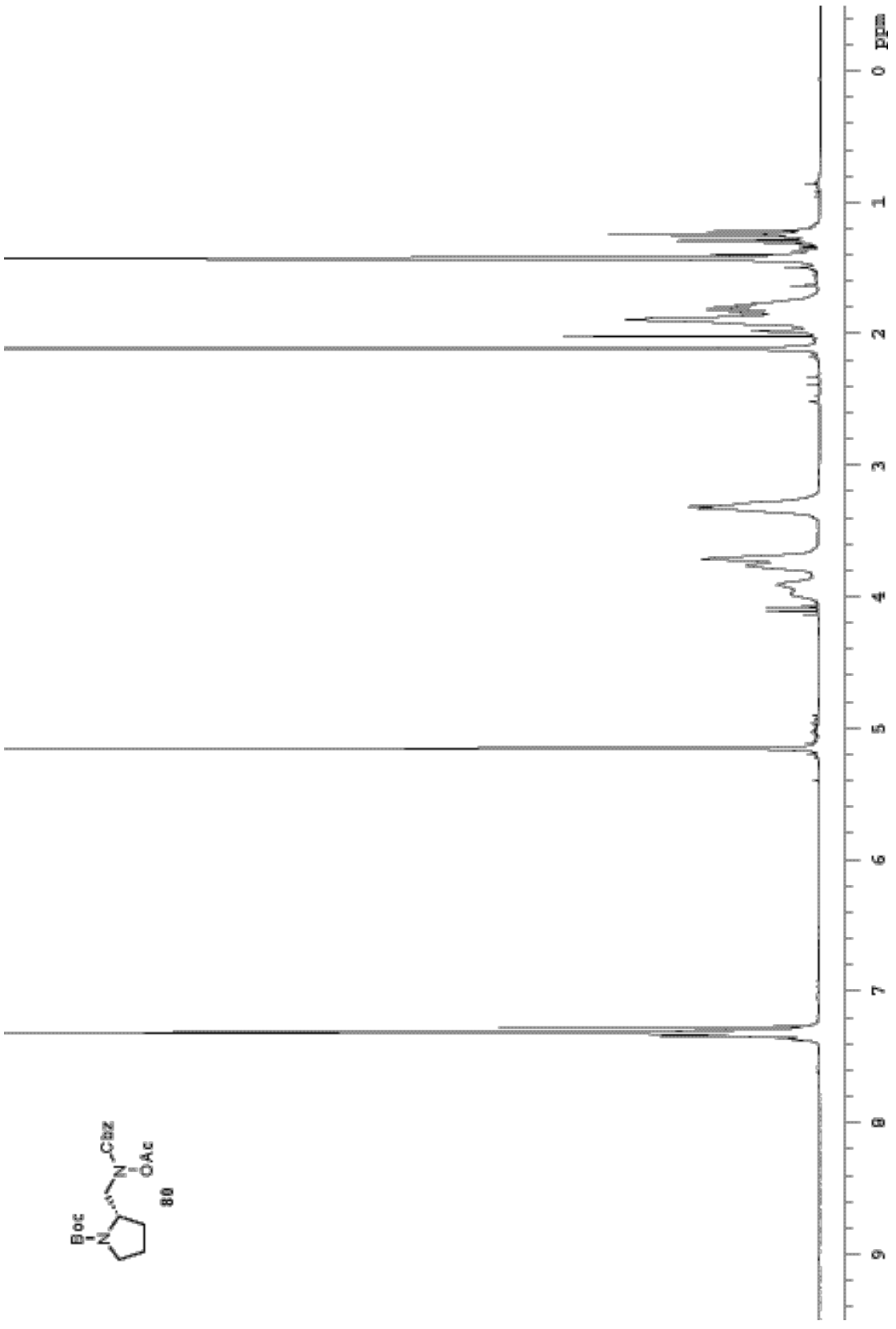
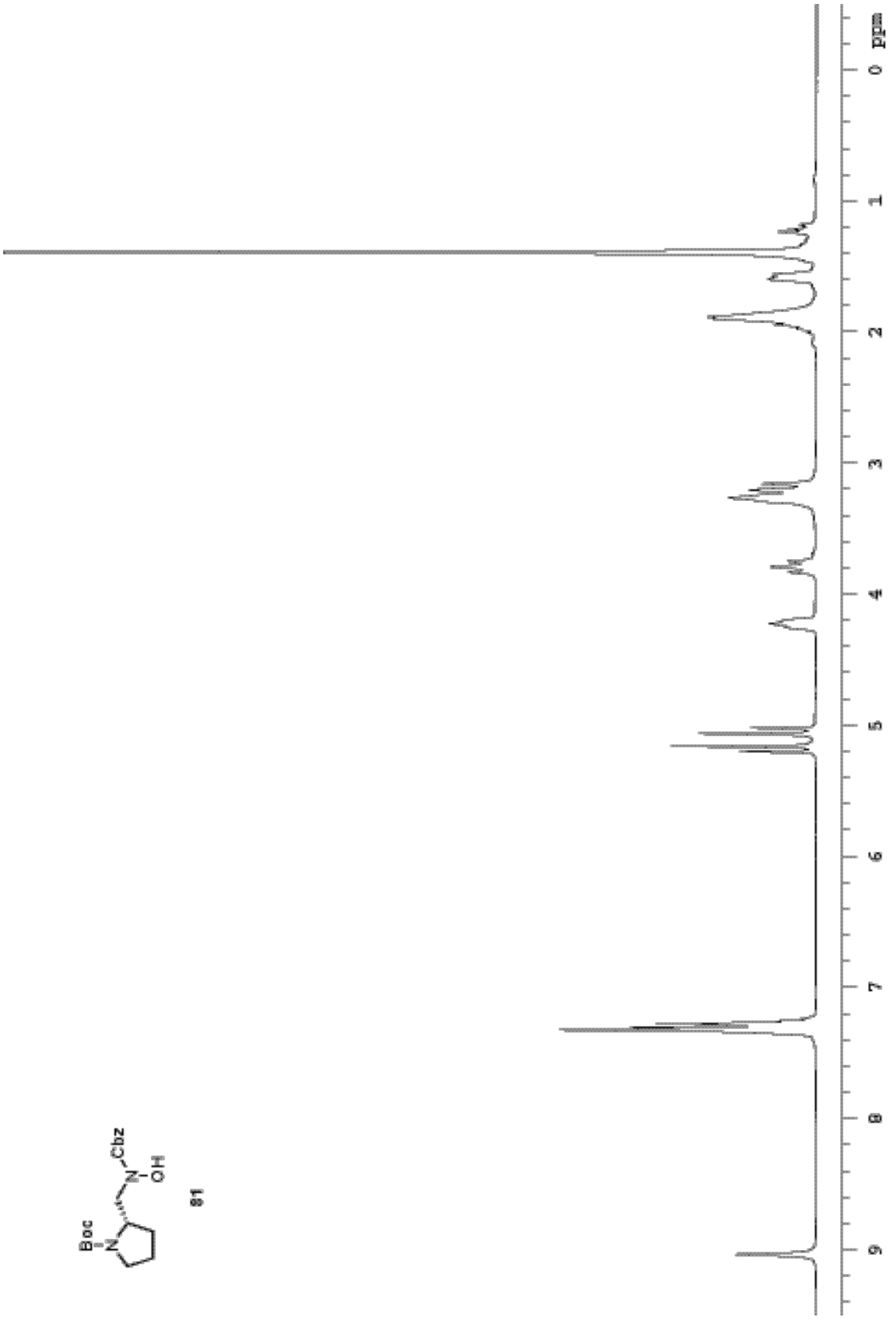
APPENDIX A

SELECTED SPECTRAL DATA

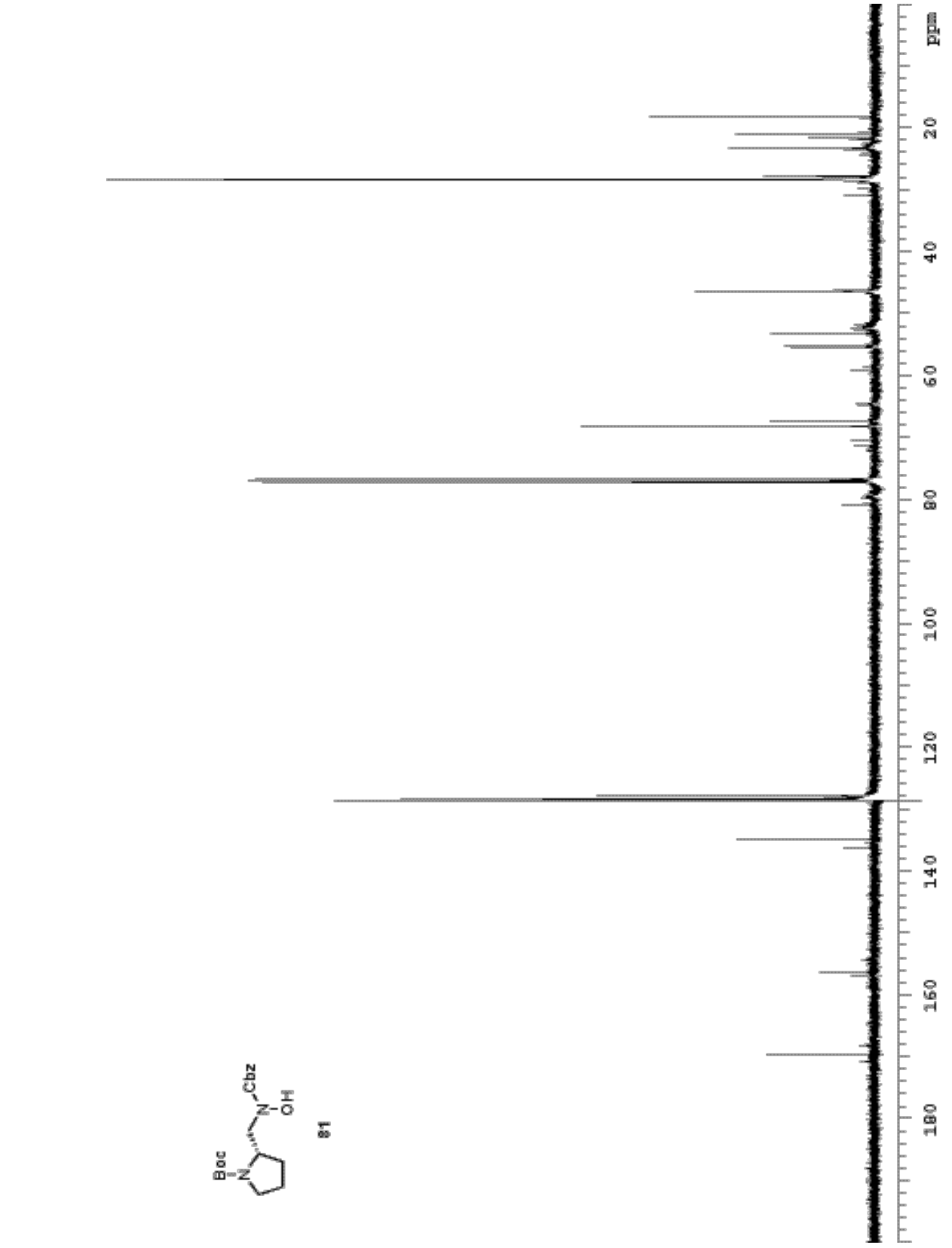


^{1}H NMR spectrum for *N*-acetoxy prolinamide **80** ($CDCl_3$)

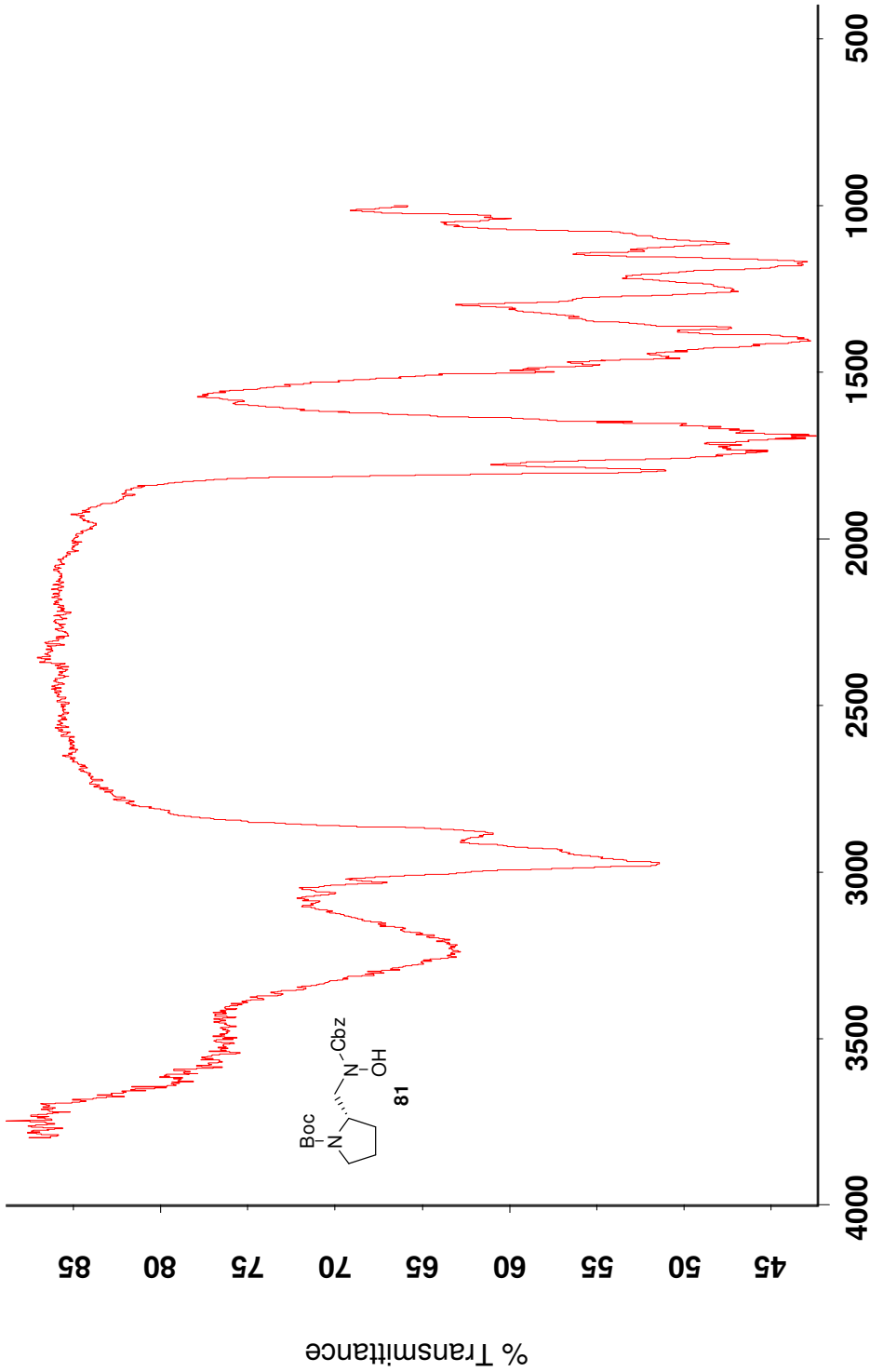
IR spectrum for *N*-acetoxy prolinamide **80**



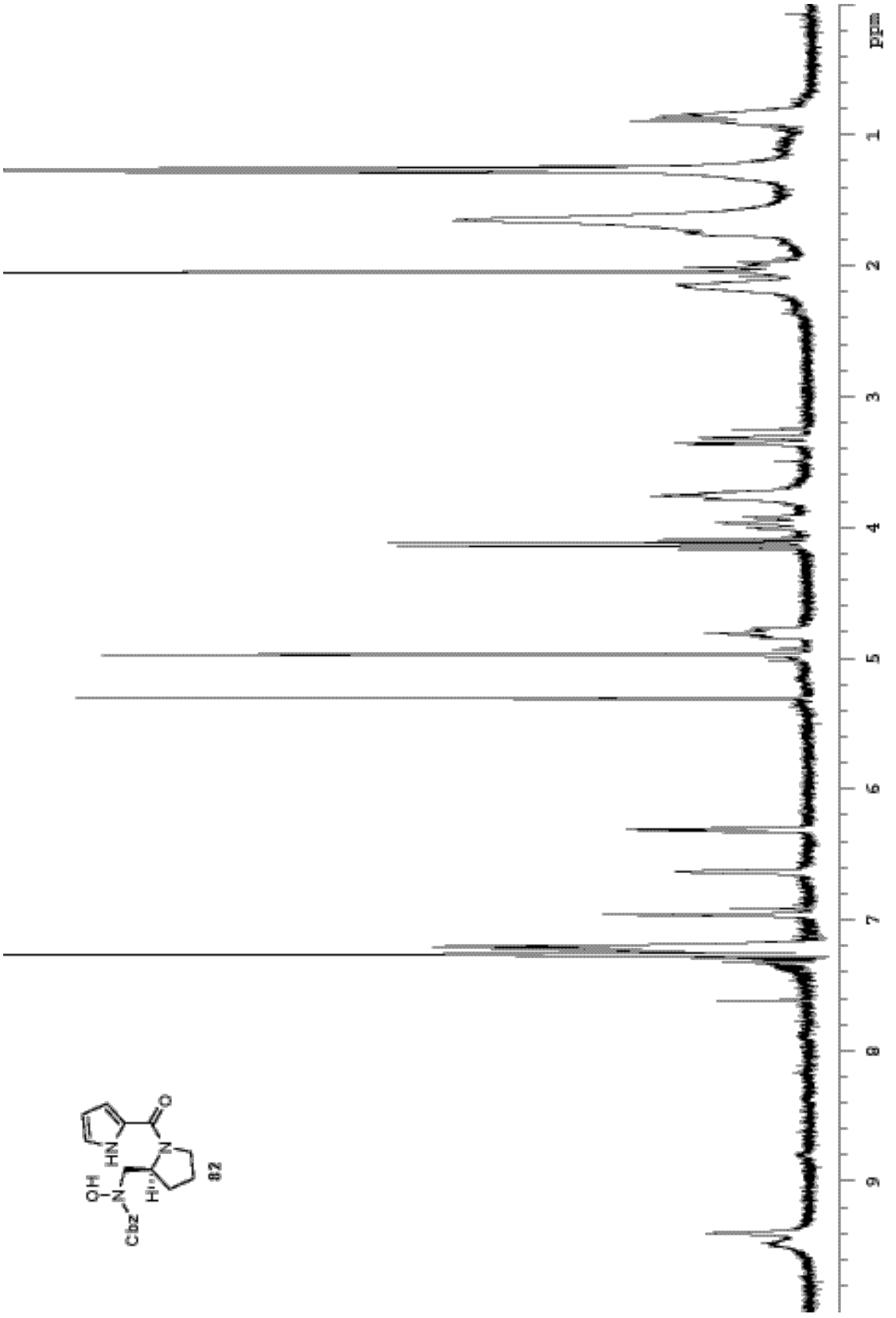
^{1}H NMR spectrum for *N*-hydroxy prolinamide **81** ($CDCl_3$)



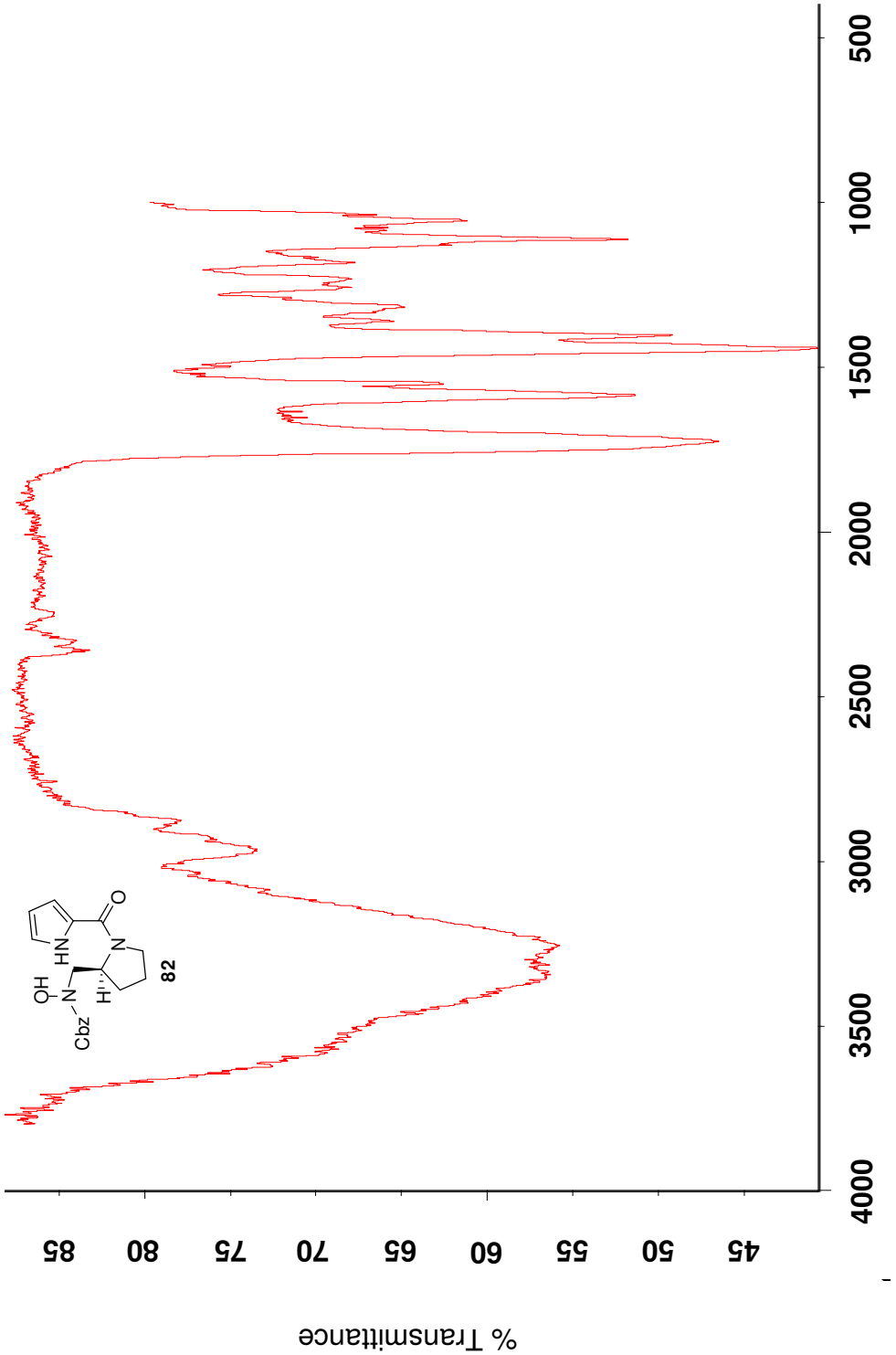
^{13}C NMR spectrum for *N*-hydroxy prolinamide **81** ($CDCl_3$)



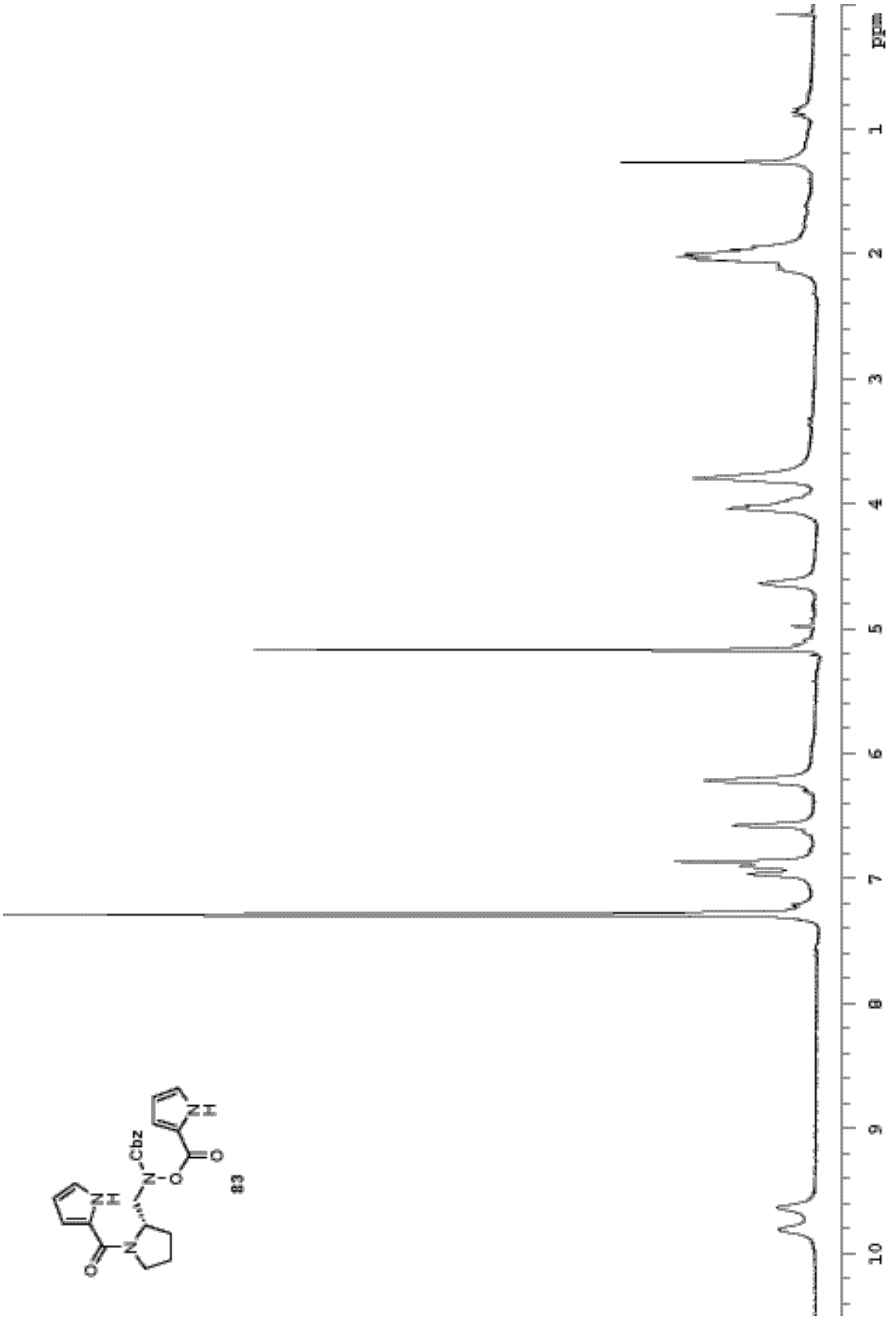
IR spectrum for *N*-hydroxy prolinamide **81**



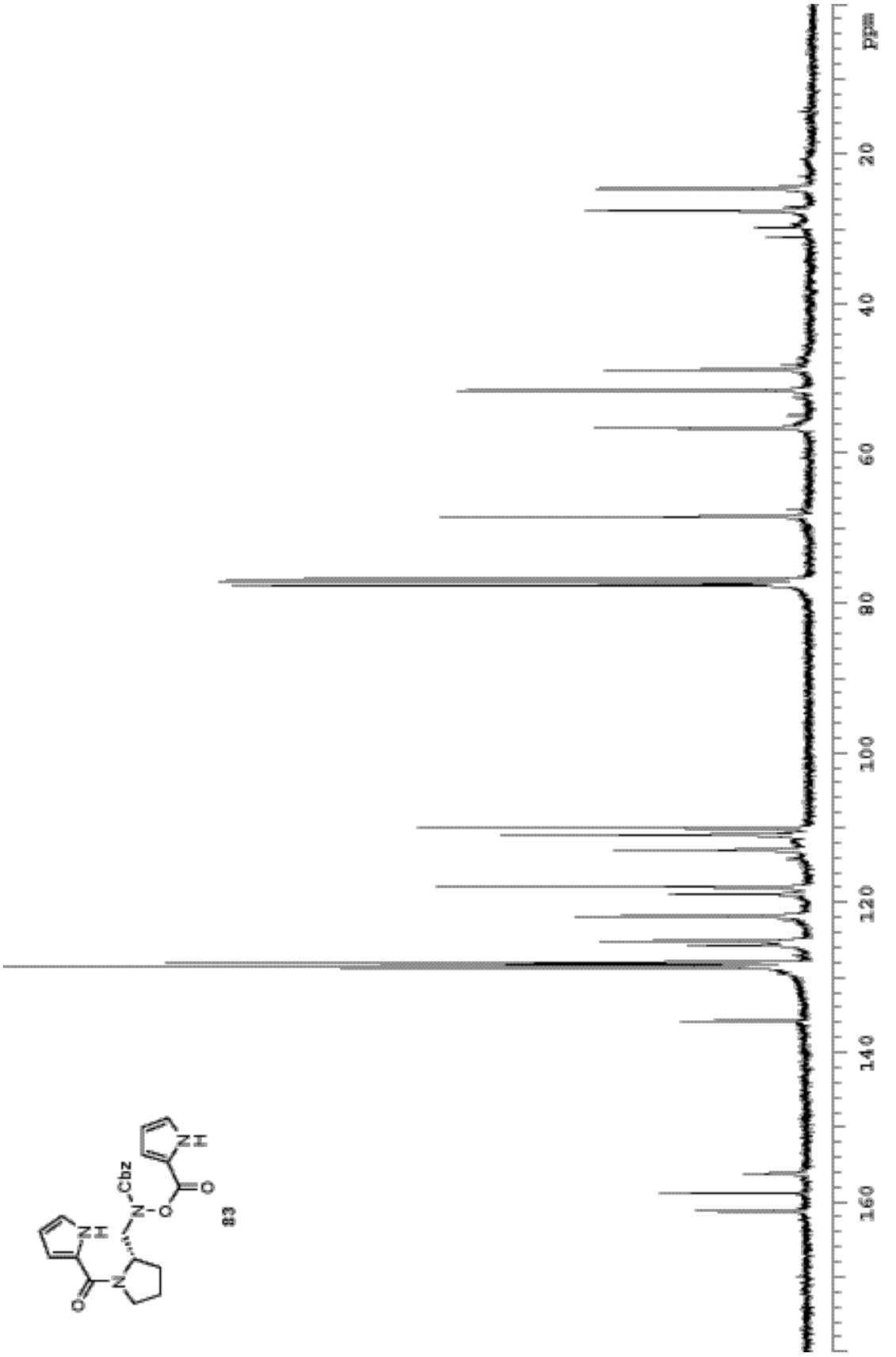
^{1}H NMR spectrum for *N*-hydroxy carbamate **82** ($CDCl_3$)



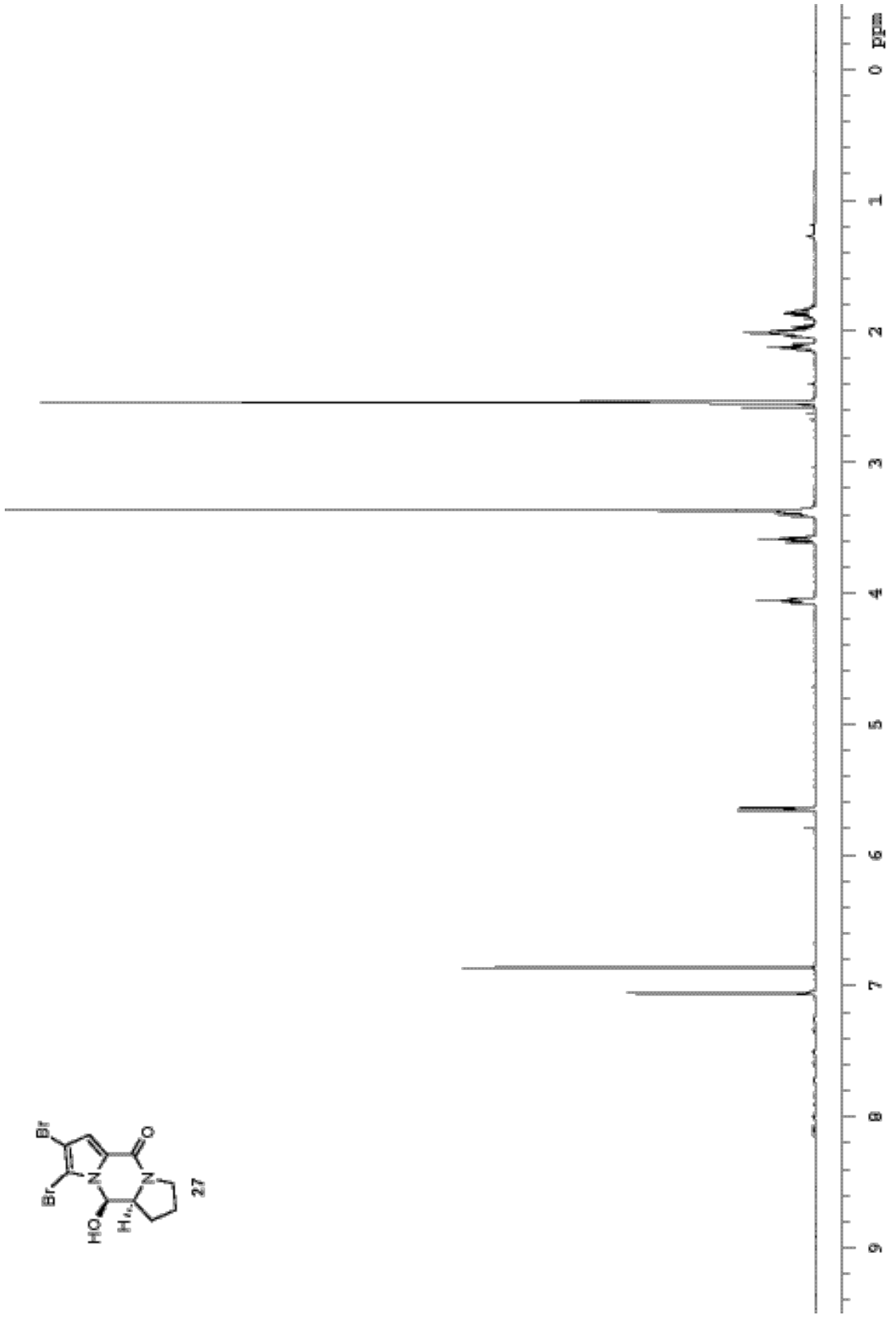
IR spectrum for *N*-hydroxy carbamate **82**



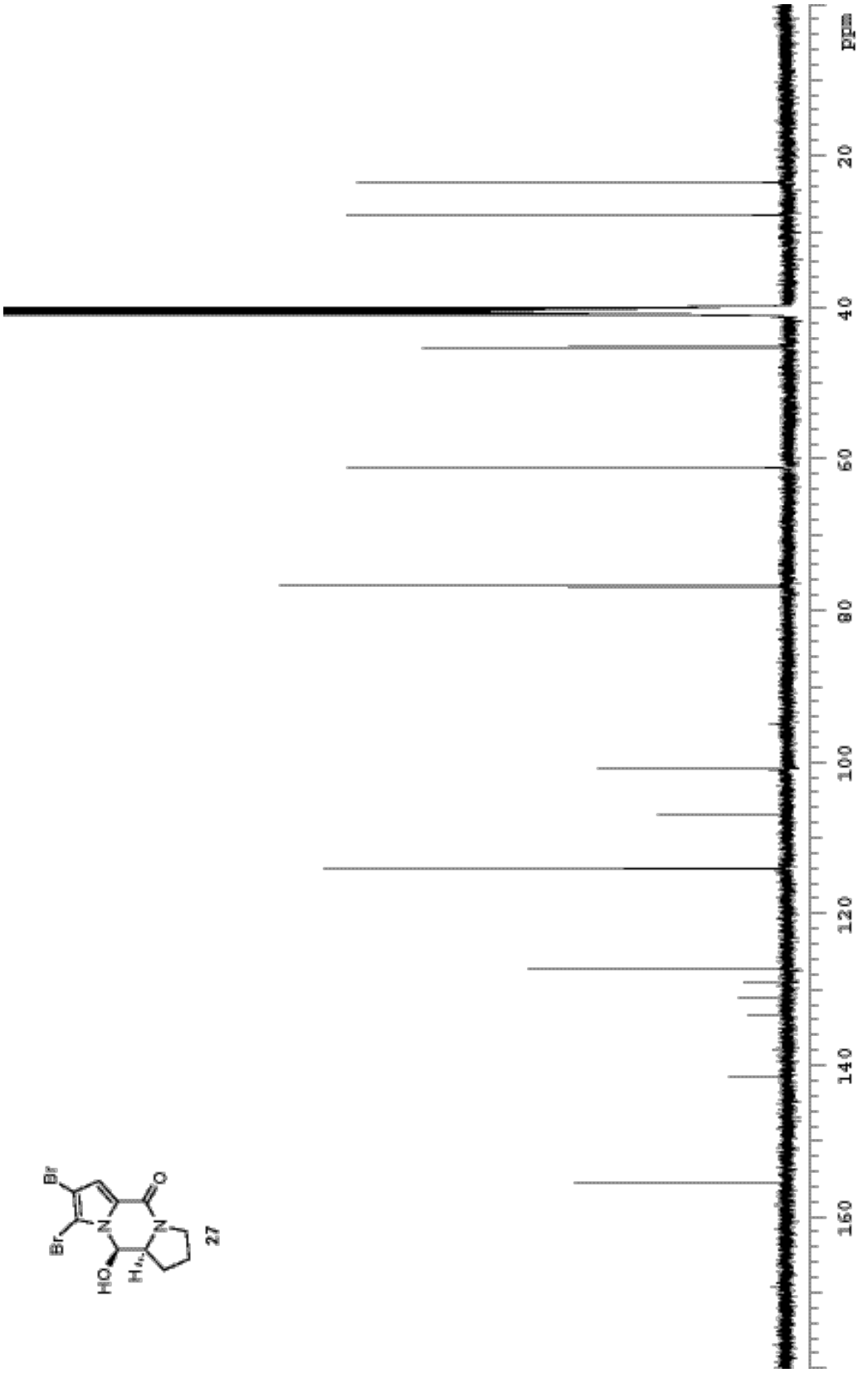
^{1}H NMR spectrum for Bis-acylated compound **83** ($CDCl_3$)



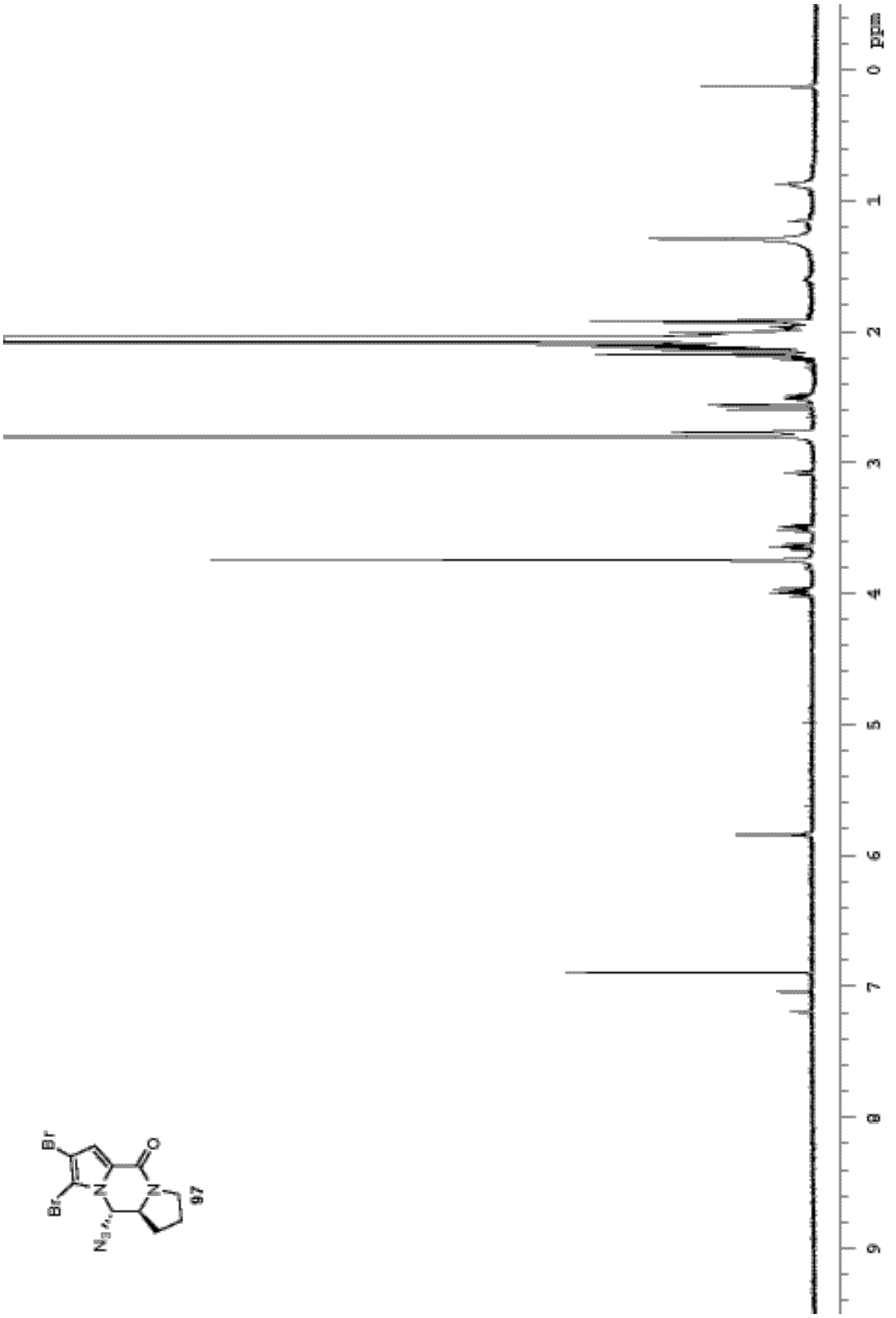
^{13}C NMR spectrum for Bis-acylated compound **83** ($CDCl_3$)



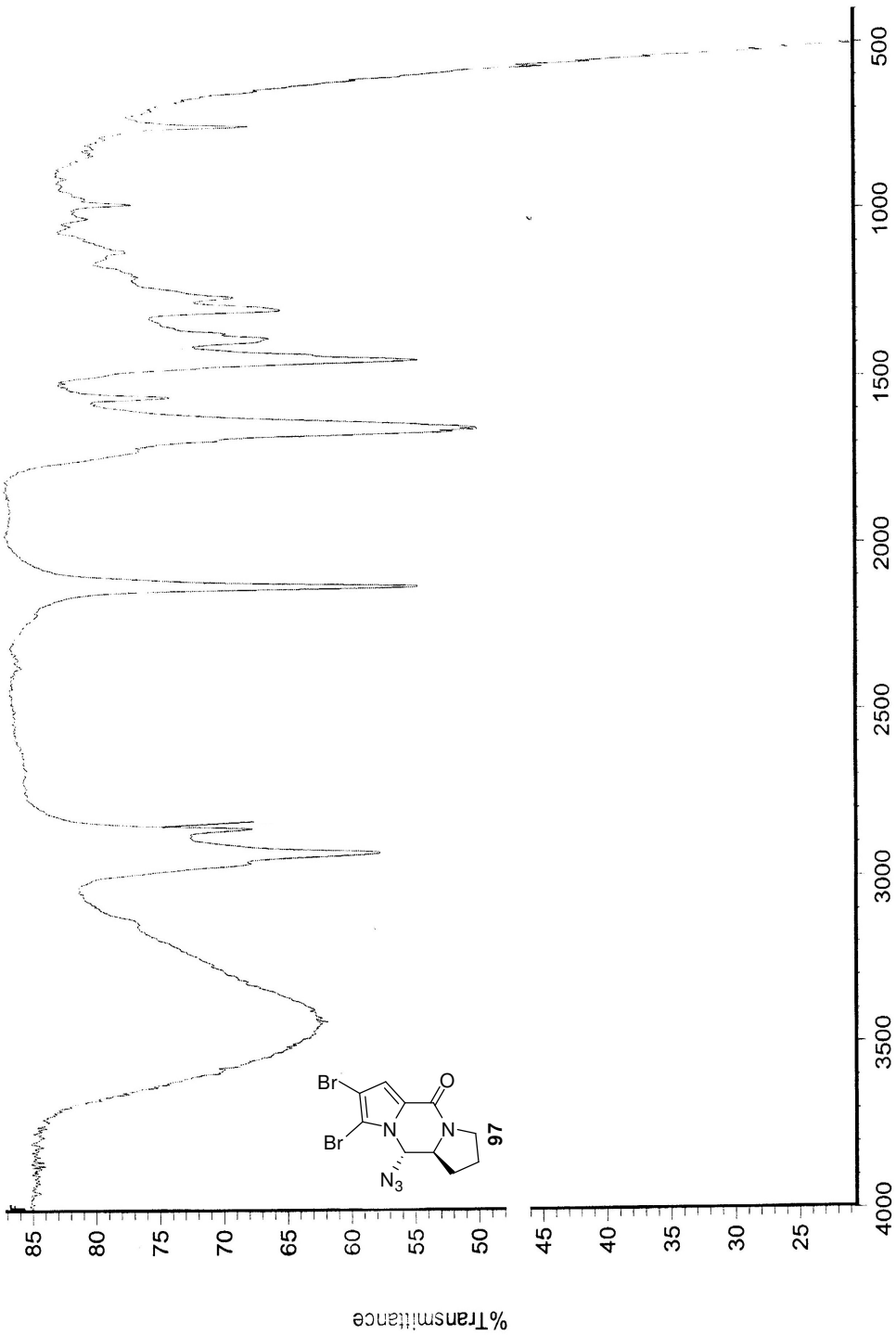
^{1}H NMR spectrum for *N,O*-hemiacetal **27** (d_6-dmso)



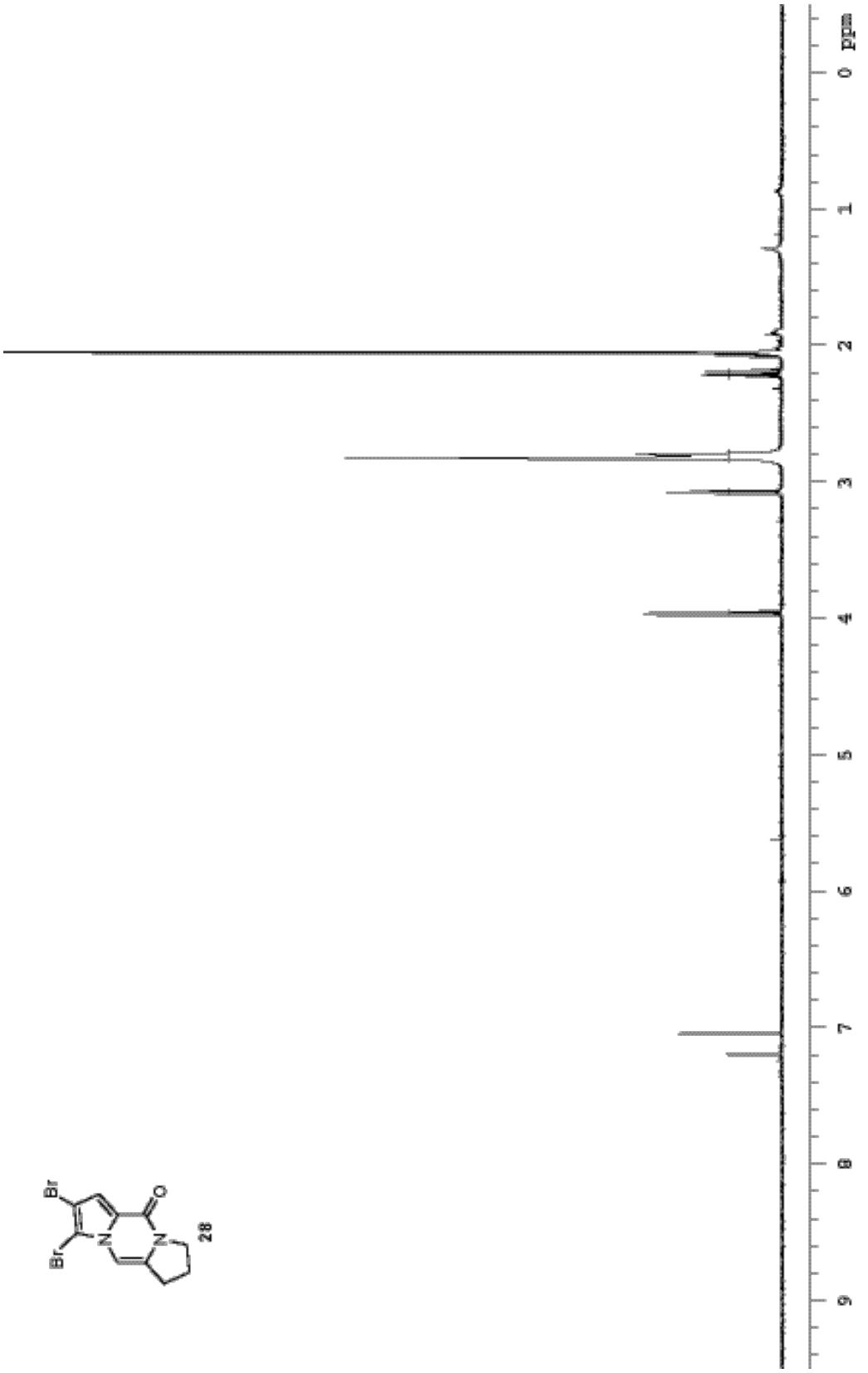
^{13}C NMR spectrum for *N,O*-hemiacetal **27** (d_6-dmso)



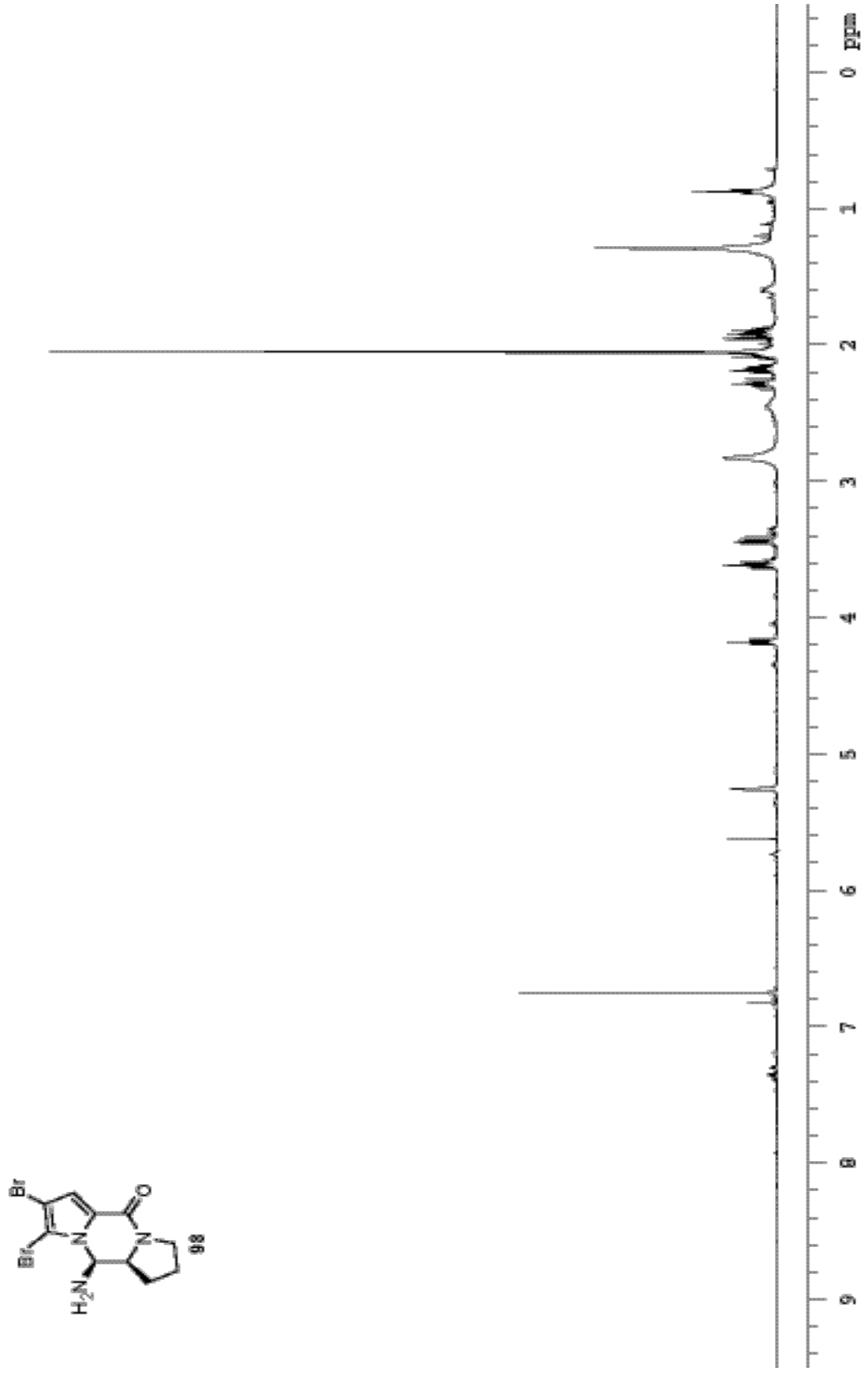
^{1}H NMR spectrum for azide **97** (acetone-d_6)



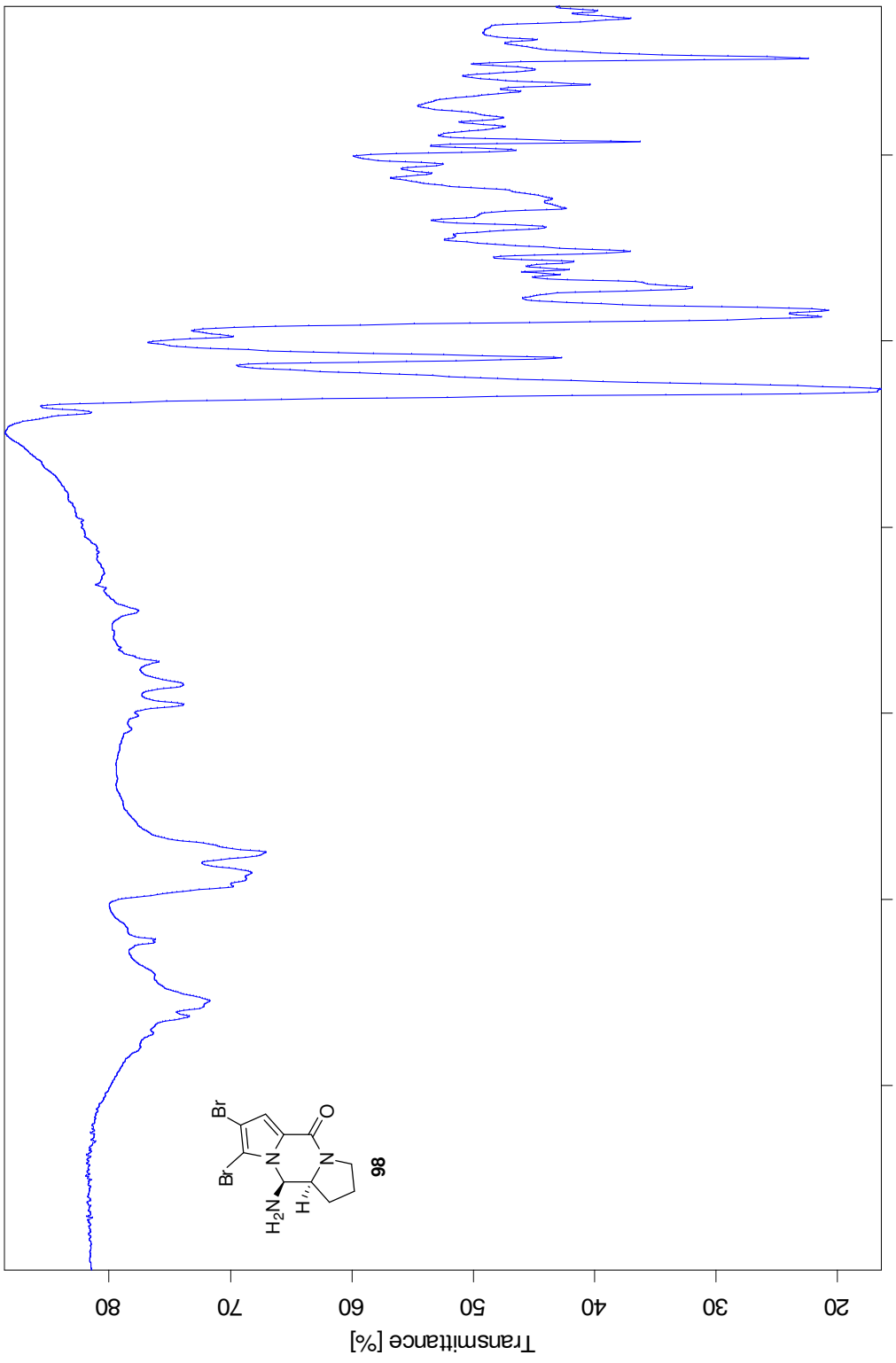
IR spectrum for azide **97**



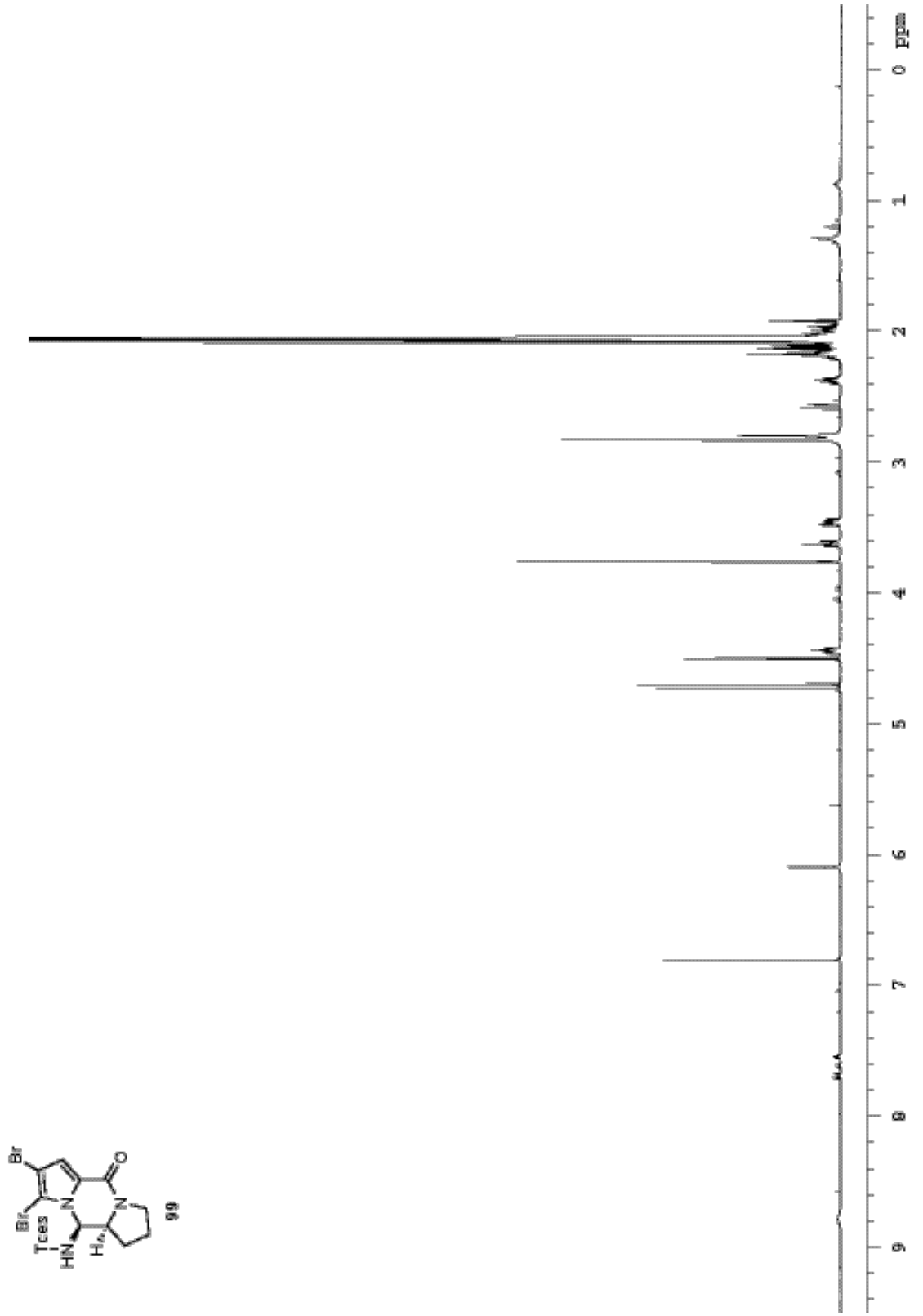
^{1}H NMR spectrum for enamide **28** (acetone-d_6)



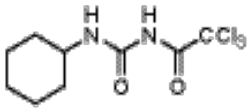
^{1}H NMR spectrum for hemi-aminal **98** (acetone-d_6)



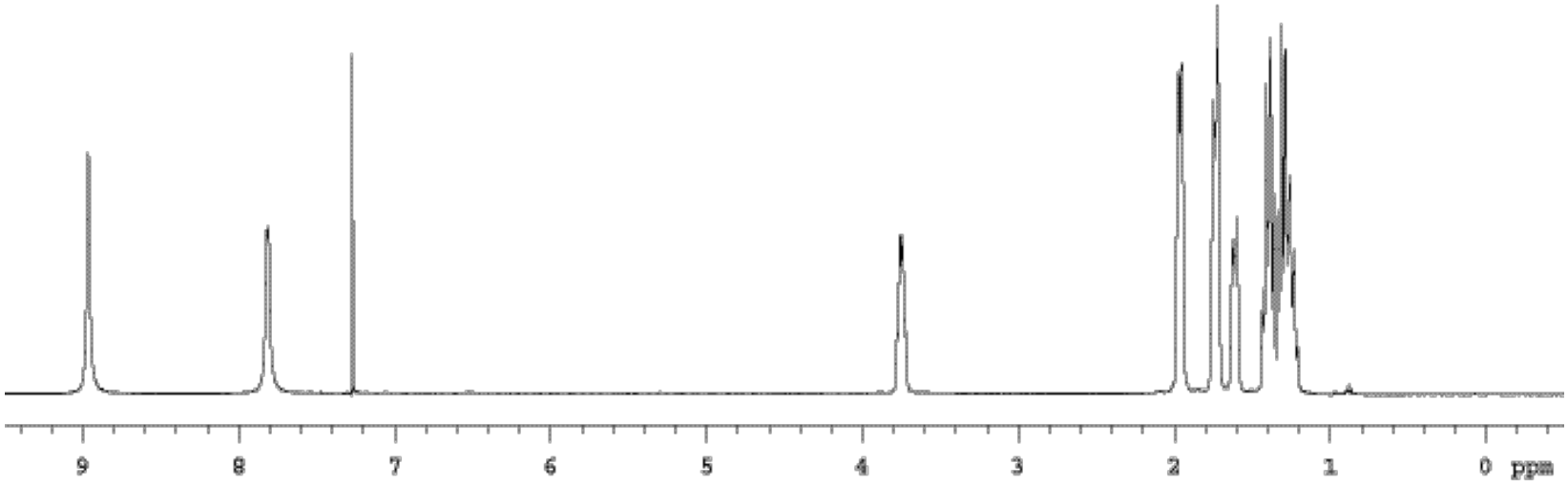
IR spectrum for hemi-aminal **98**



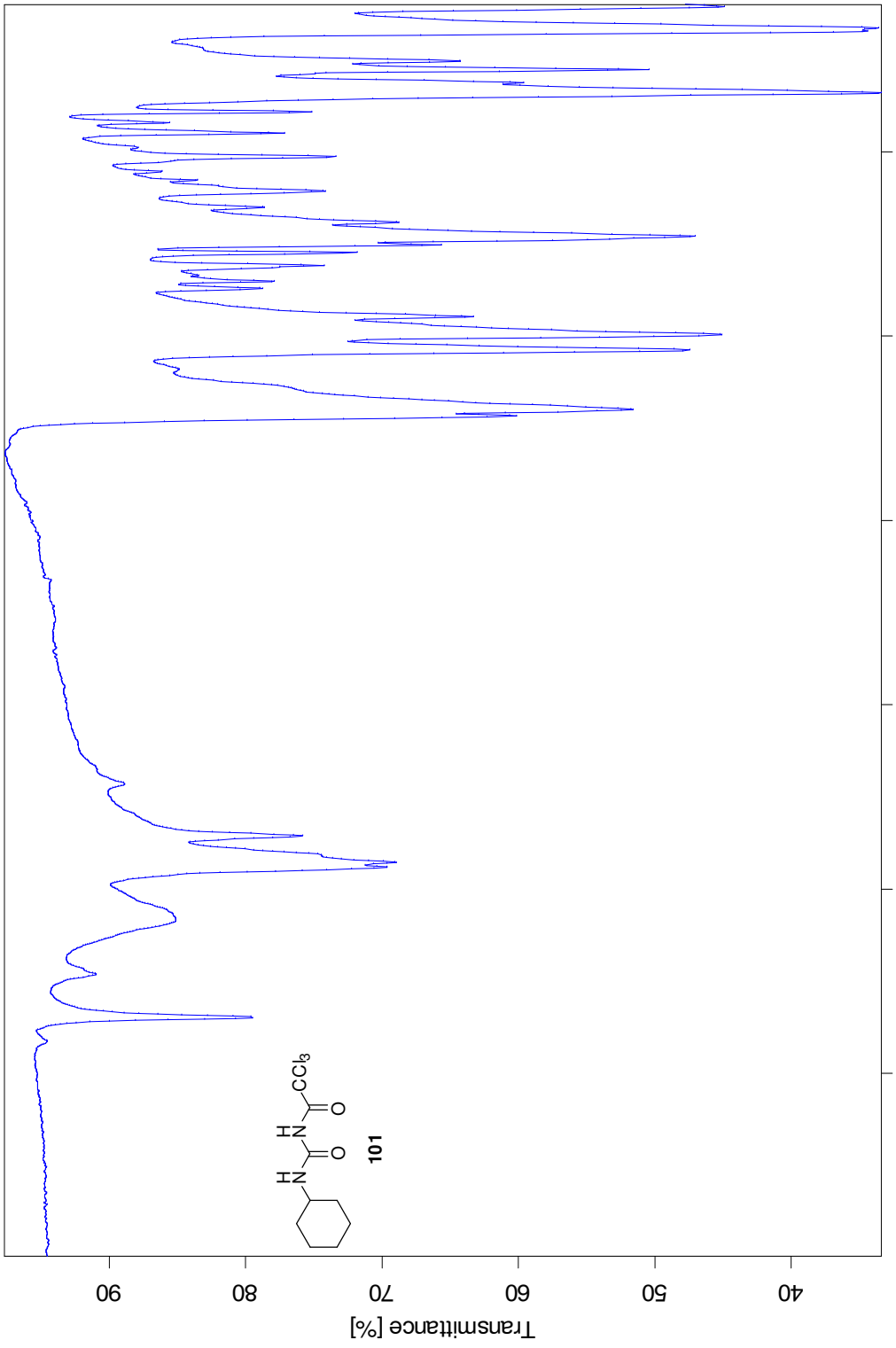
^{1}H NMR spectrum for *N*-Tces hemi-aminal **99** (acetone-d_6)



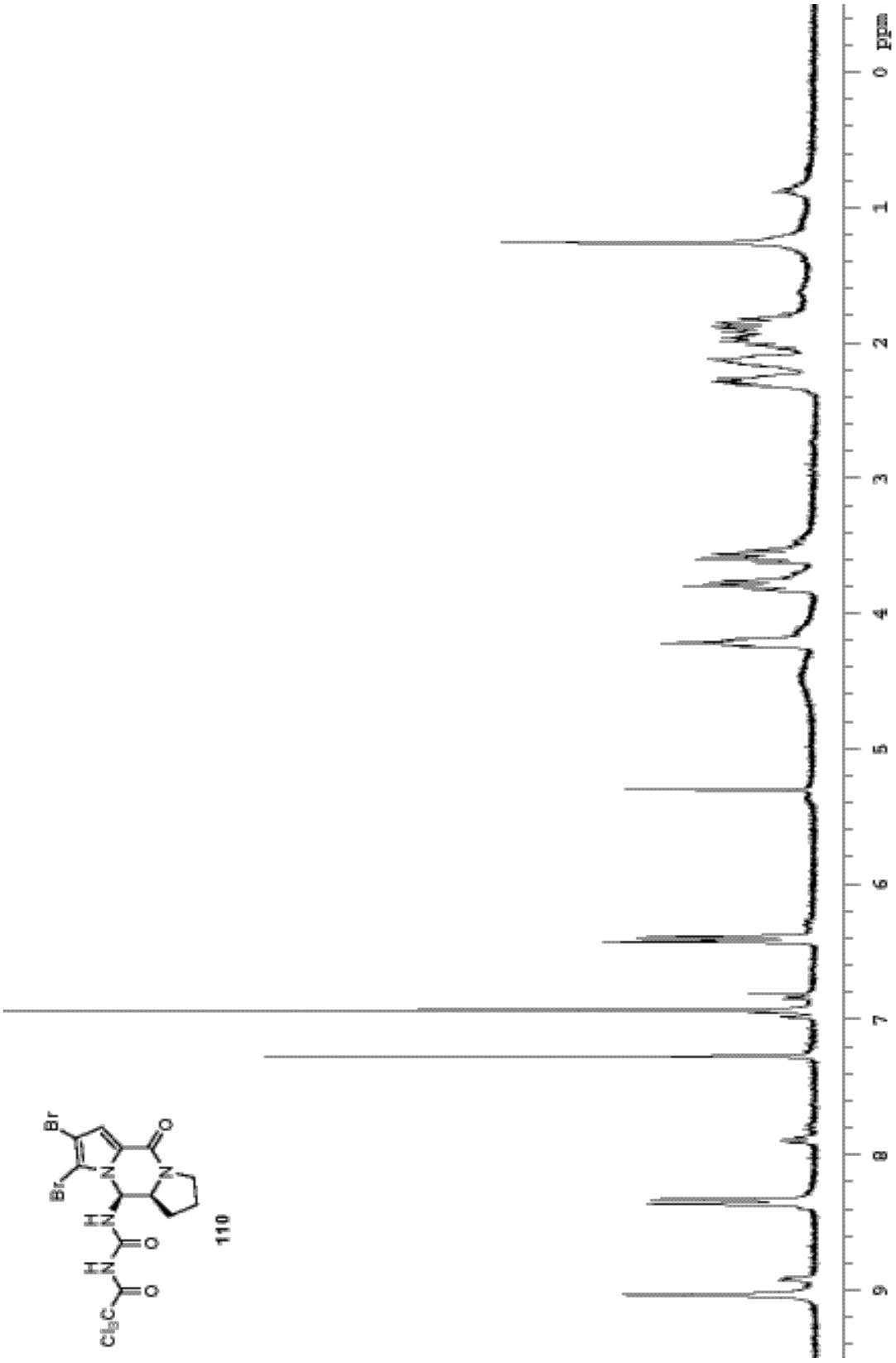
101



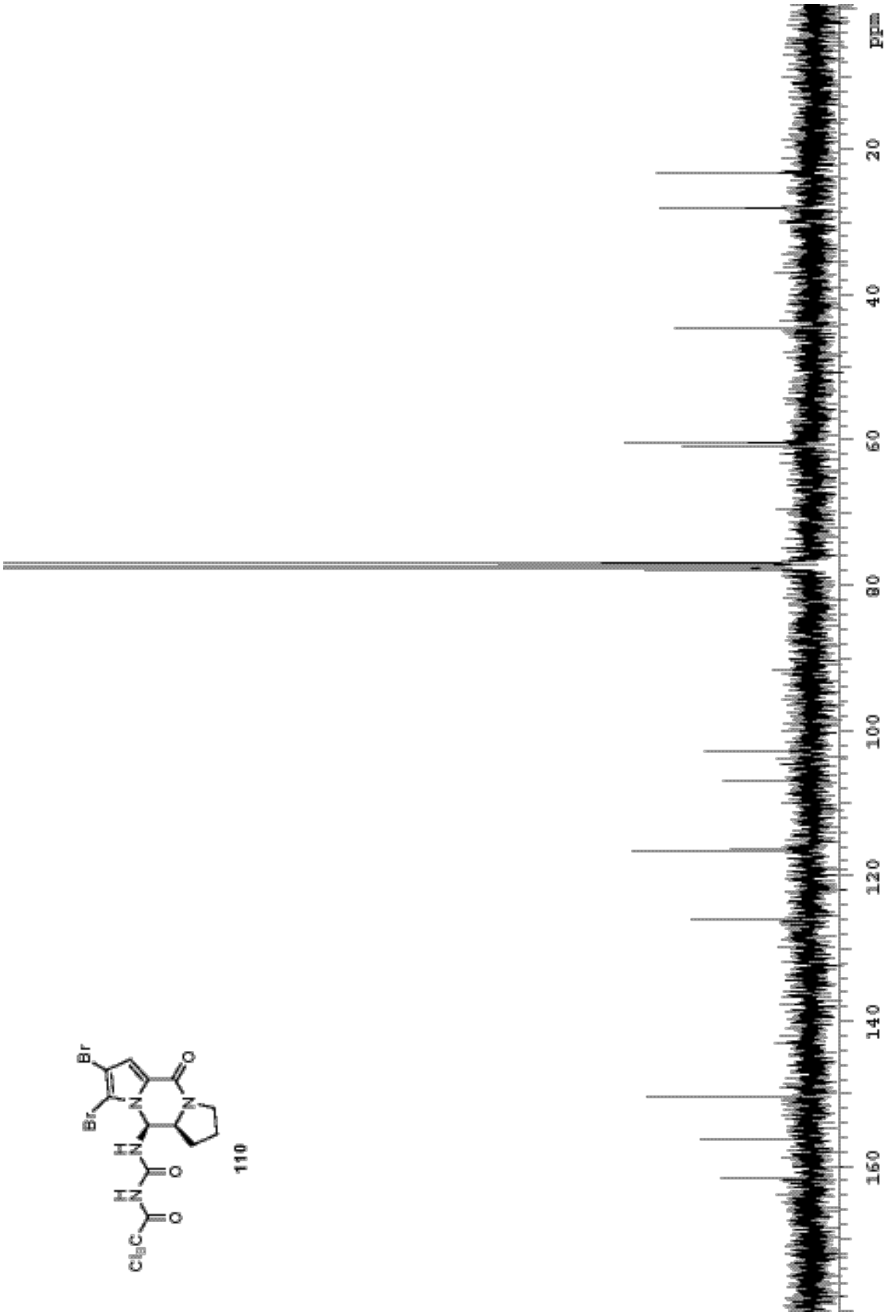
^{1}H NMR spectrum for *N*-trichloroacetyl-*N'*-cyclohexylurea **101** ($CDCl_3$)



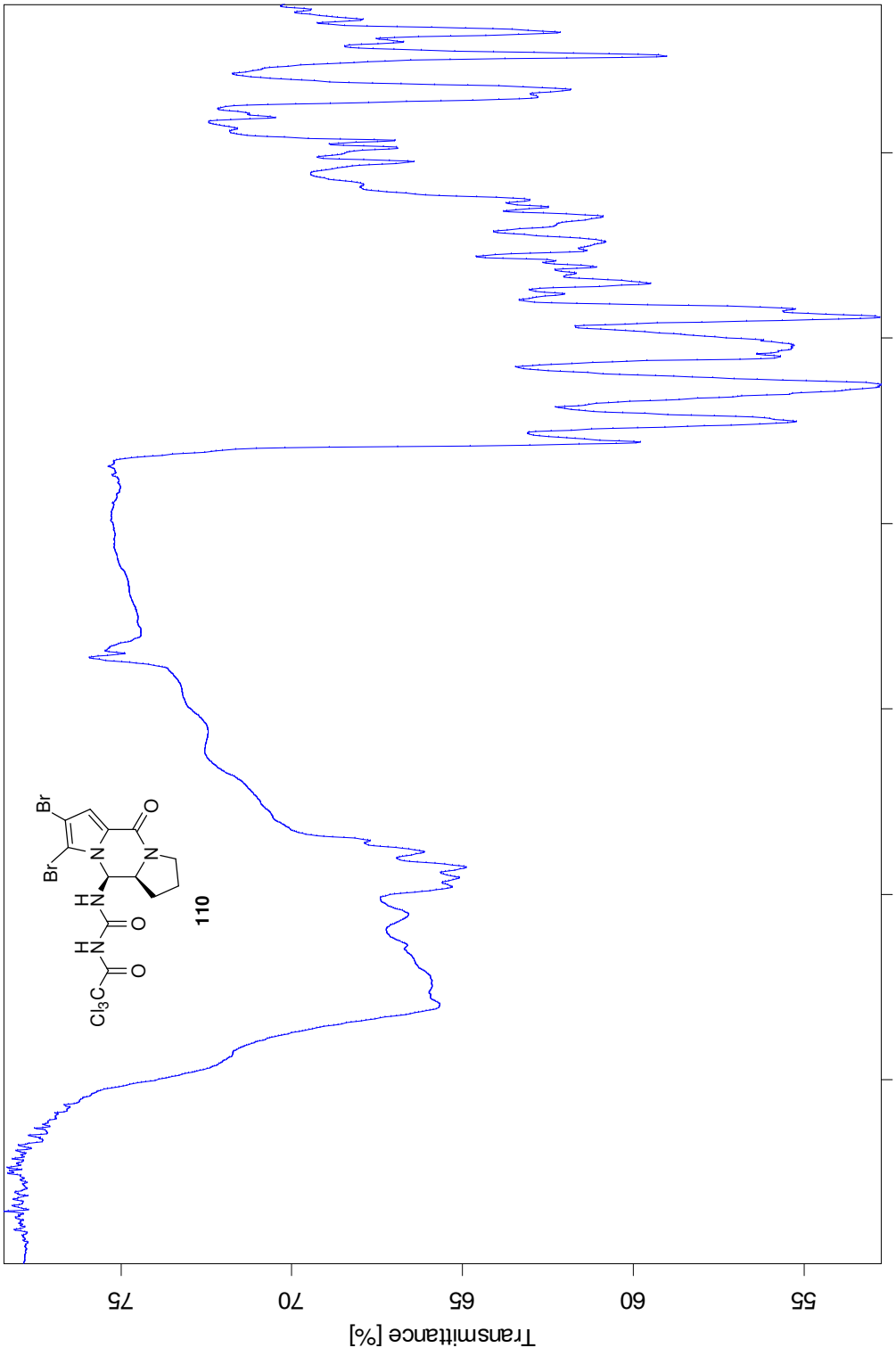
IR spectrum for *N*-trichloroacetyl-*N'*-cyclohexylurea **101**



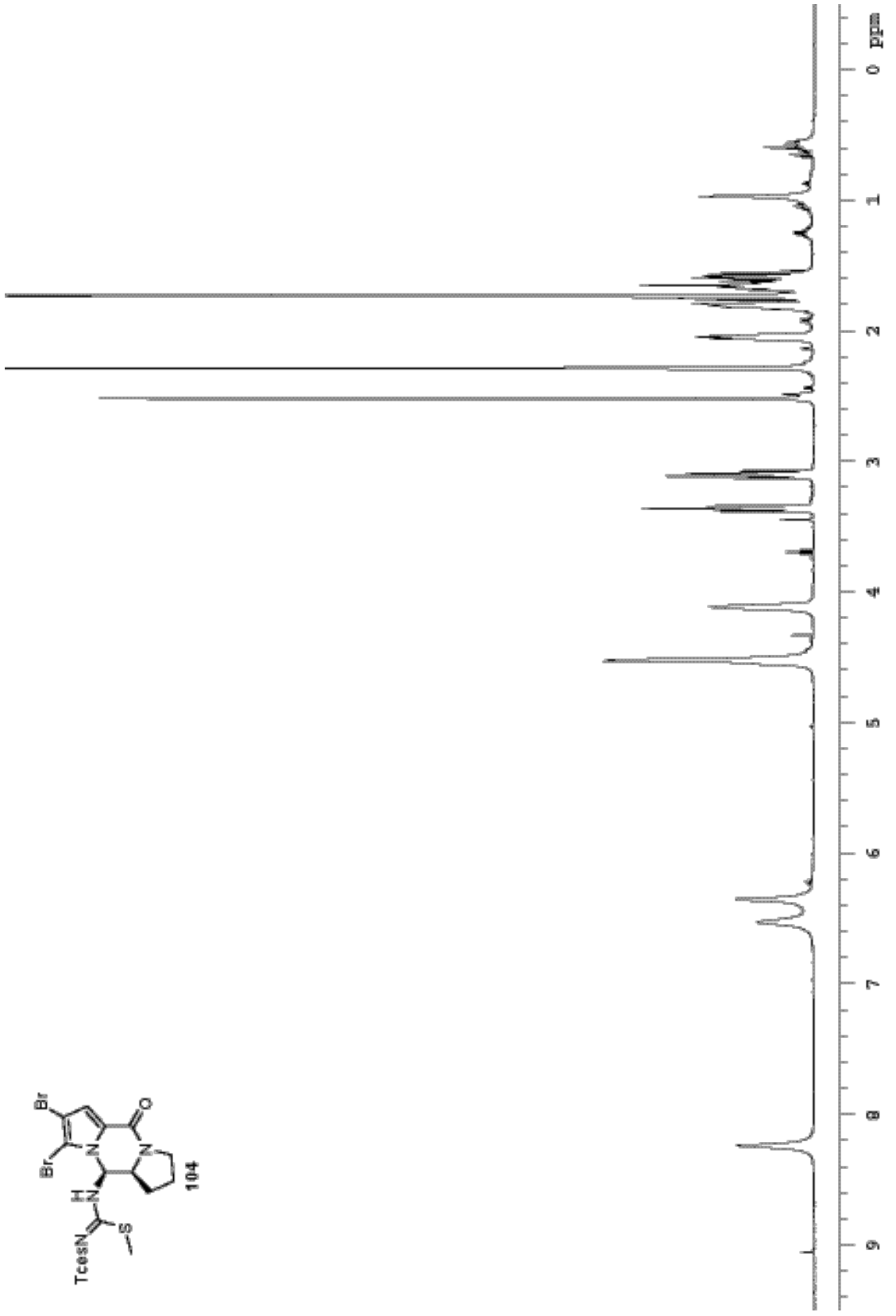
^{1}H NMR spectrum for imidine **110** ($CDCl_3$)



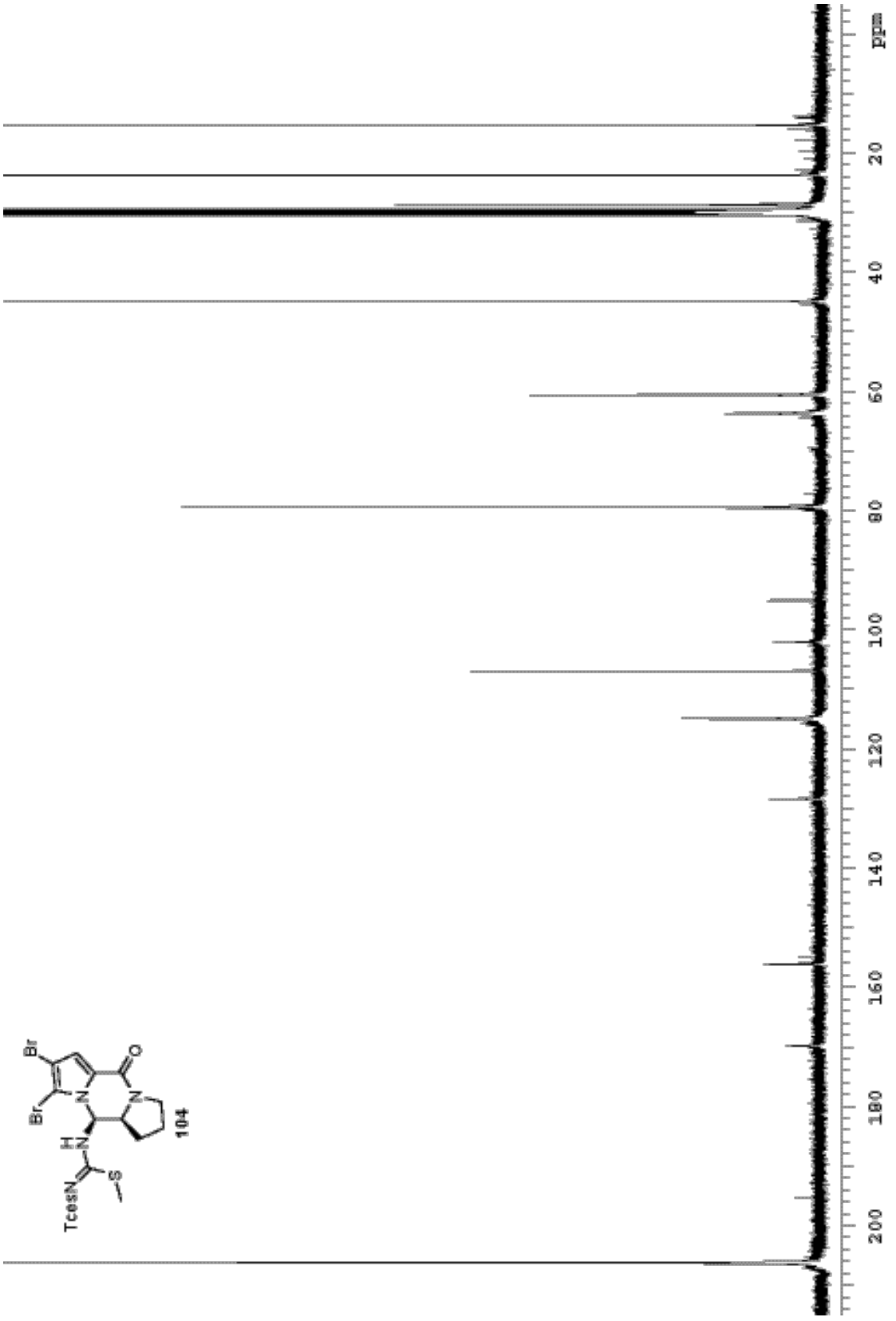
^{13}C NMR spectrum for imidine **110** ($CDCl_3$)



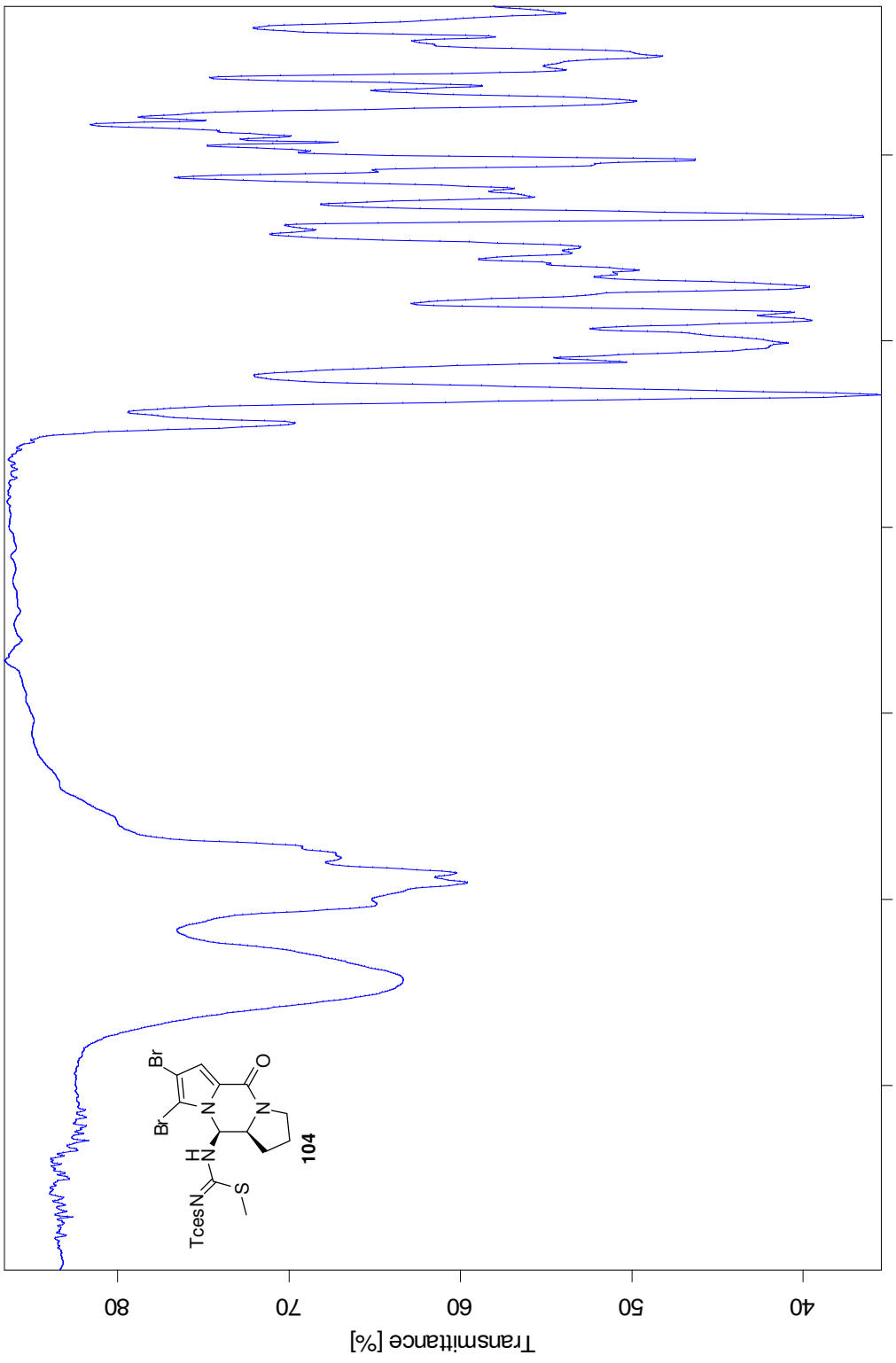
IR spectrum for imidine **110**



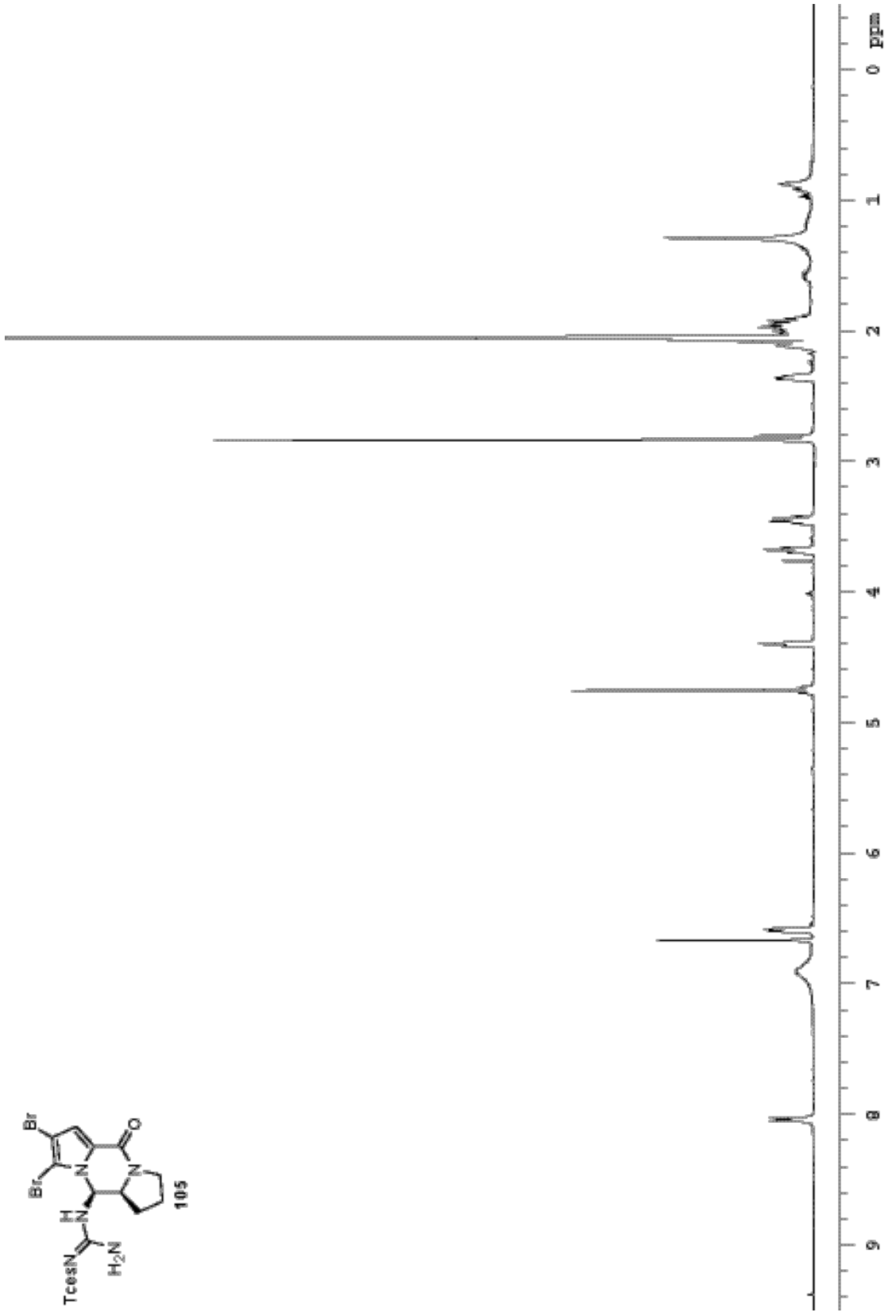
^{1}H NMR spectrum for isothiourea **104** (acetone-d_6)



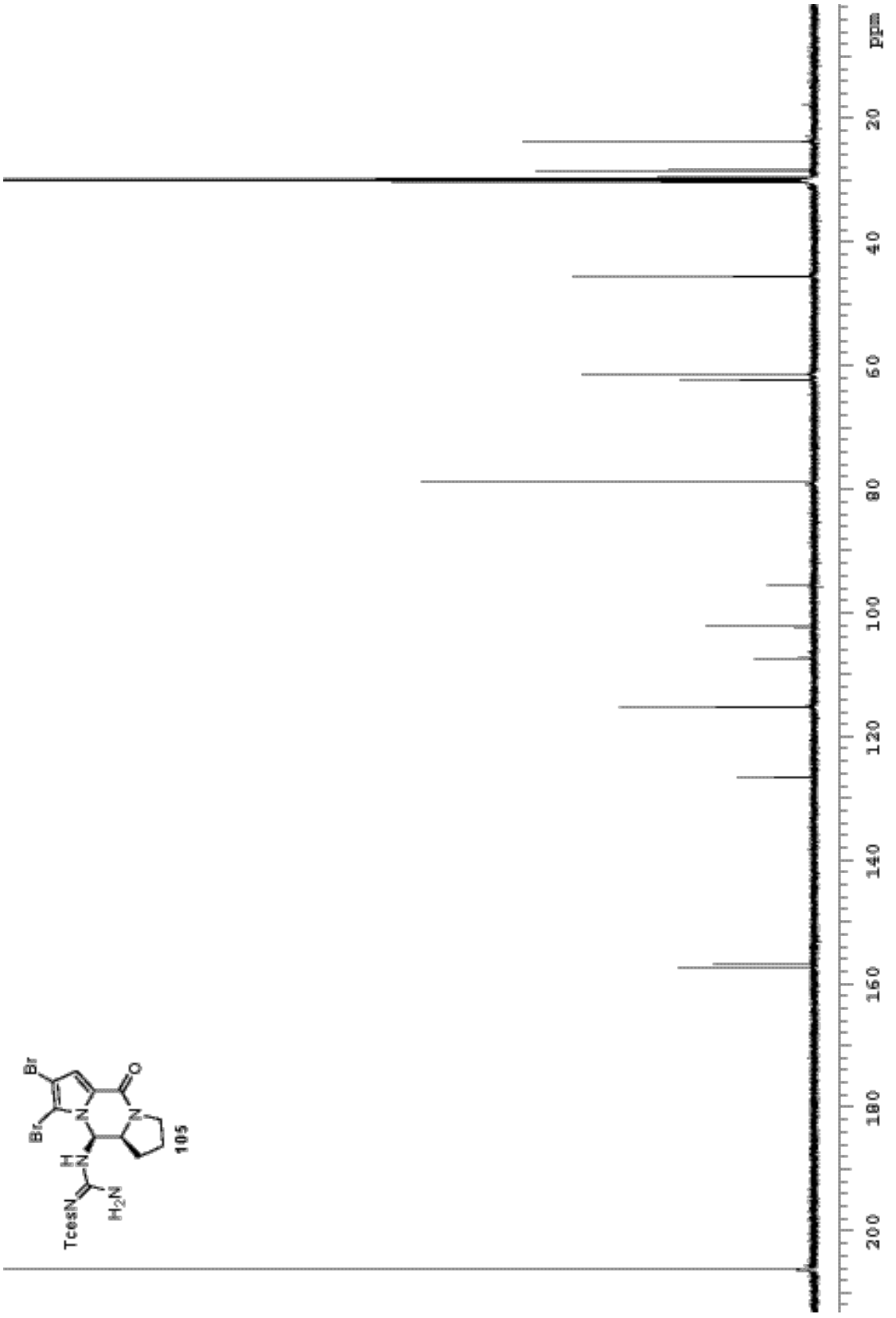
^{1}H NMR spectrum for isothiourea **104** (acetone-d_6)



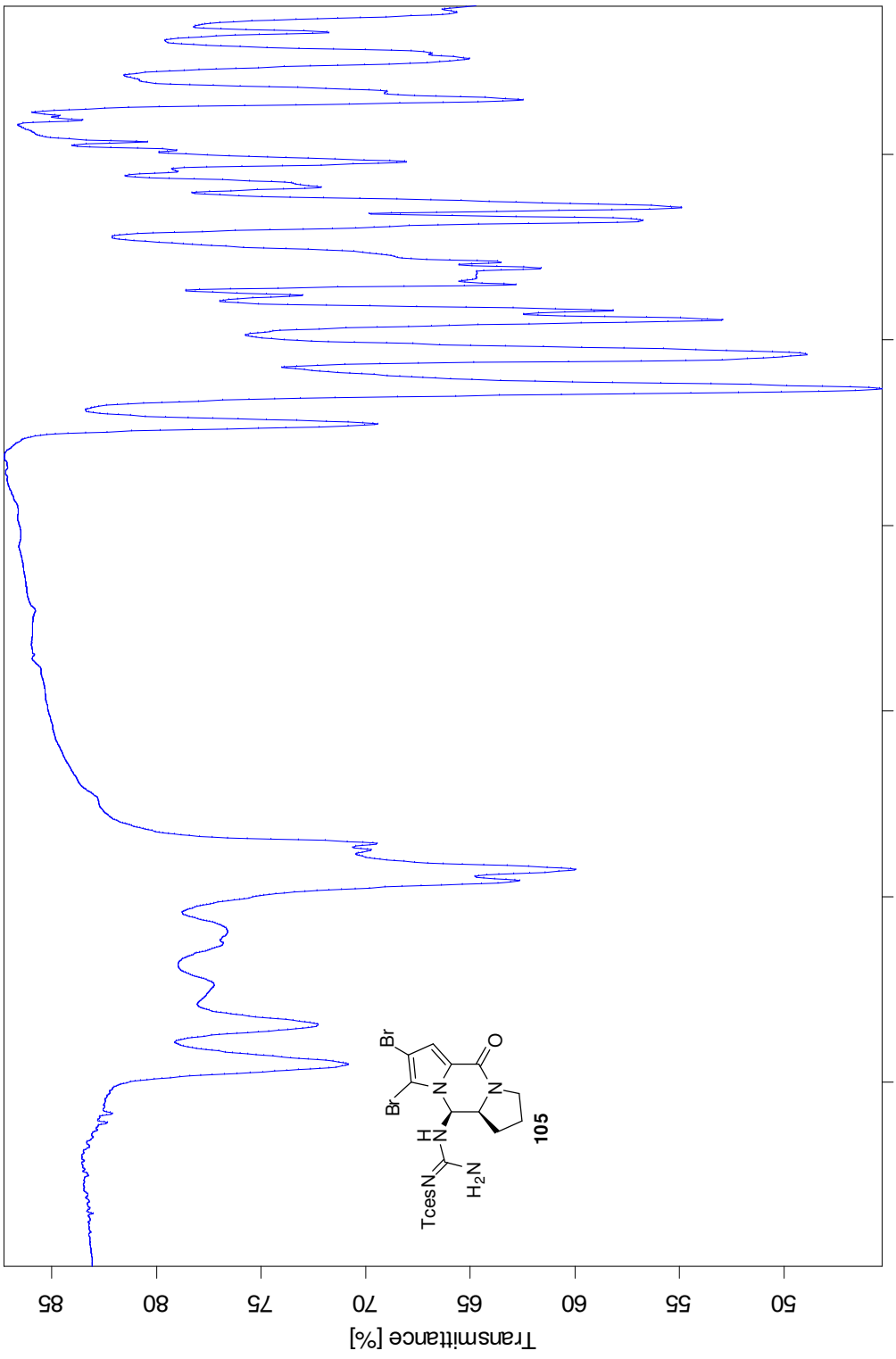
IR spectrum for isothiourea **104**



^{1}H NMR spectrum for guanidine **105** (acetone-d_6)



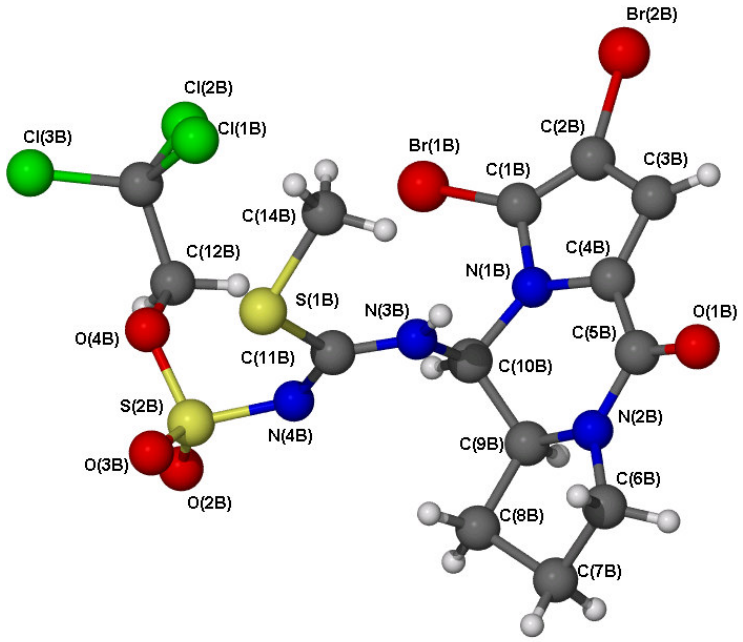
^{1}H NMR spectrum for guanidine **105** (acetone-d_6)

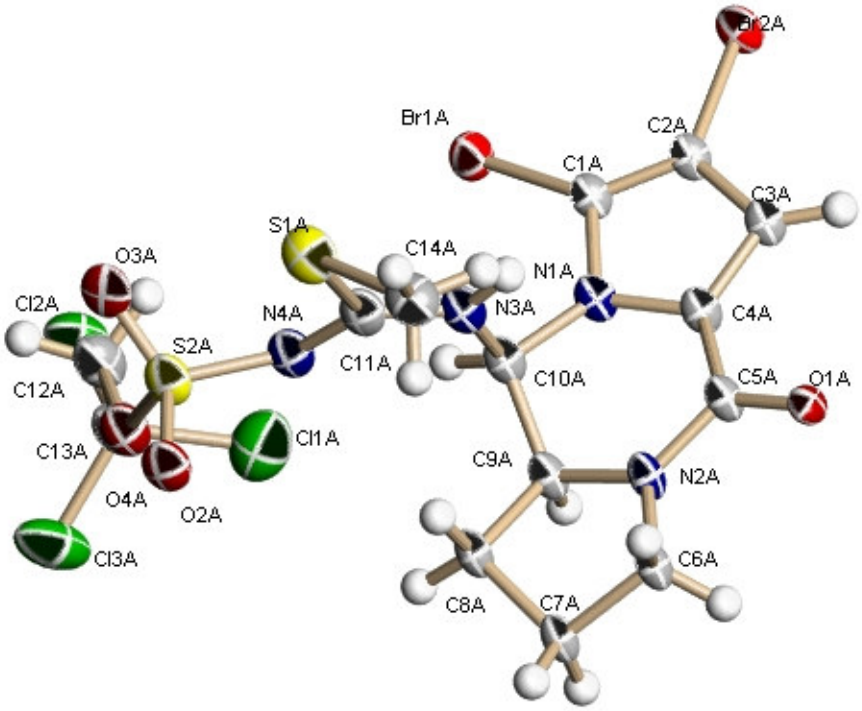


IR spectrum for guanidine **105**

APPENDIX B

CRYSTALLOGRAPHIC DATA


Br(2B)
Cl(2B)
Cl(1B)
Cl(3B)
C(2B)
Br(1B)
C(1B)
C(3B)
C(14B)
C(12B)
C(4B)
N(1B)
S(1B)
N(3B)
O(1B)
O(4B)
C(5B)
C(11B)
C(10B)
S(2B)
N(2B)
N(4B)
C(9B)
O(3B)
C(6B)
O(2B)
C(8B)
C(7B)


Br2A
Br1A
C2A
C1A
C3A
S1A
C14A
N1A
O3A
Cl2A
N4A
N3A
C4A
S2A
C11A
C10A
C5A
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O4A
O2A
C6A
Cl3A
C8A
C7A

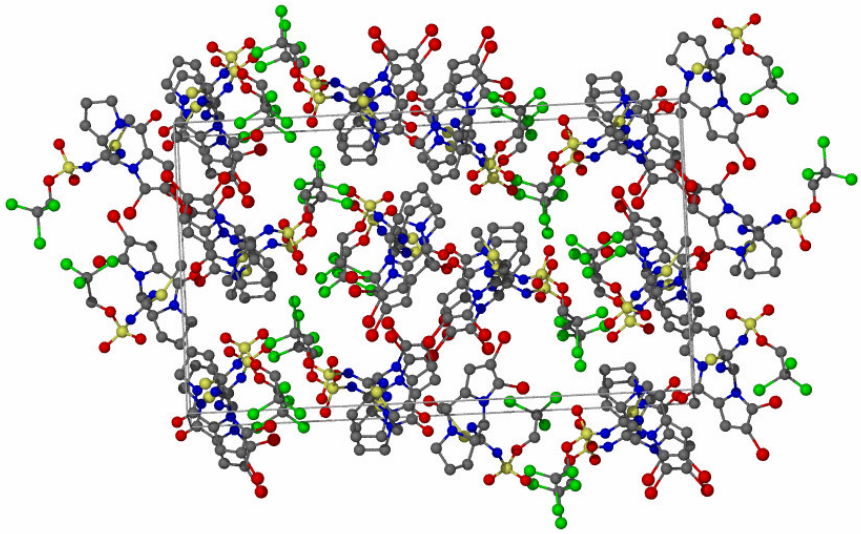
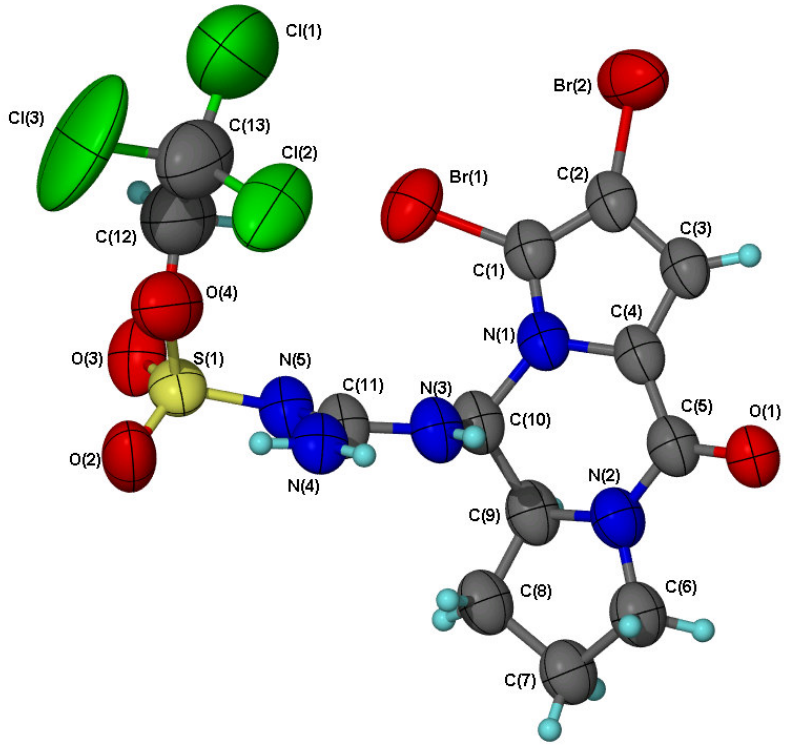


Table 8. Crystal data and structure refinement for isothiourea **104**.

Identification code	dr53a	
Empirical formula	C14 H15 Br2 Cl3 N4 O4 S2	
Formula weight	633.59	
Temperature	110(2) K	
Wavelength	1.54184 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 8.634(4) Å	α= 90°.
	b = 17.310(7) Å	β= 90°.
	c = 29.935(12) Å	γ = 90°.
Volume	4474(3) $Å^3$	
Z	8	
Density (calculated)	1.881 Mg/m^3	
Absorption coefficient	9.913 mm^{-1}	
F(000)	2496	
Crystal size	0.14 x 0.12 x 0.02 mm^3	
Theta range for data collection	2.95 to 59.02°.	
Index ranges	-7<=h<=8, -19<=k<=18, -22<=l<=33	
Reflections collected	15968	
Independent reflections	5728 [R(int) = 0.2080]	
Completeness to theta = 59.02°	91.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.8264 and 0.3375	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5728 / 569 / 523	
Goodness-of-fit on F^2	1.066	
Final R indices [I>2sigma(I)]	R1 = 0.0736, wR2 = 0.1238	
R indices (all data)	R1 = 0.1731, wR2 = 0.1660	
Absolute structure parameter	-0.03(6)	
Largest diff. peak and hole	0.695 and -0.655 e.$Å^{-3}$	



Comments

¼ molecule of C_6H_{14} was found in the asymmetric unit. The hexane was disordered between multiple positions and refinement of its positional parameters was not possible. Finally the electron density of the hexane was subtracted from the over data by employing the routine SQUEEZE in PLATON 2006 (see A.L.Spek (2005) PLATON, A Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands; A.L.Spek, J.Appl.Cryst. 2003, 36, 7-13). After the subtraction the molecule was refined normally.

Table 9. Crystal data and structure refinement for guanidine **105**.

Identification code	dr57	
Empirical formula	C14.50 H15.50 Br2 Cl3 N5 O4 S	
	C13 H12 Br2 Cl3 N5 O4 S ¼(C6H14)	
Formula weight	622.05	
Temperature	110(2) K	
Wavelength	0.71073 Å	
Crystal system	Trigonal	
Space group	P3(1)21	
Unit cell dimensions	a = 18.533(13) Å	α= 90°.
	b = 18.533(13) Å	β= 90°.
	c = 25.13(2) Å	γ = 120°.
Volume	7474(10) $Å^3$	
Z	12	
Density (calculated)	1.659 Mg/m^3	
Absorption coefficient	3.688 mm^{-1}	
F(000)	3678	
Crystal size	0.50 x 0.10 x 0.10 mm^3	
Theta range for data collection	2.06 to 25.00°.	
Index ranges	-22<=h<=21, -22<=k<=22, -29<=l<=29	
Reflections collected	51706	
Independent reflections	8676 [R(int) = 0.2213]	
Completeness to theta = 25.00°	99.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7093 and 0.2600	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	8676 / 312 / 507	
Goodness-of-fit on F^2	1.014	
Final R indices [I>2sigma(I)]	R1 = 0.0724, wR2 = 0.1725	
R indices (all data)	R1 = 0.1732, wR2 = 0.2221	
Absolute structure parameter	0.01(2)	
Largest diff. peak and hole	0.786 and -0.496 e.$Å^{-3}$	

APPENDIX C

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